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NEWS	6	Oct 22	Over 1 million reactions added to CASREACT
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NEWS	13	Nov 30	Files VETU and VETB to have open access
NEWS	14	Dec 10	WPINDEX/WPIDS/WPIX New and Revised Manual Codes for 2002
NEWS	15	Dec 10	DGENE BLAST Homology Search
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NEWS	18	Dec 17	New fields for DPCI
NEWS	19	Dec 19	CAS Roles modified
NEWS	20	Dec 19	1907-1946 data and page images added to CA and CAplus
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NEWS	22	Jan 25	Searching with the P indicator for Preparations
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NEWS	24	Feb 01	DKILIT now produced by FIZ Karlsruhe and has a new update frequency

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=> s nucleotide and modified allergen
L1 2 NUCLEOTIDE AND MODIFIED ALLERGEN

=> dup remove l1
PROCESSING COMPLETED FOR L1
L2 2 DUP REMOVE L1 (0 DUPLICATES REMOVED)

=> d l2 1-2 cbib abs

L2 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine of the City University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

AB It has been detd. that allergens, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by masking the site with a compd. that prevents IgE binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the IgE-binding epitope, to eliminate IgE binding. The method allows the protein to be altered as minimally as possible, other than within the IgE-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use peanut allergens to demonstrate alteration of IgE binding sites. The crit. amino acids within each of the IgE binding epitopes of the peanut protein that are important to Ig binding have been detd. Substitution of even a single amino acid within each of the epitopes led to loss of IgE binding. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most crit. to IgE binding.

L2 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS

1993:232264 Document No. 118:232264 Ryegrass pollen allergen. Singh, Mohan Bir; Hough, Terryn; Knox, Robert Bruce; Theerakulpisut, Piyada; Smith, Penelope; Avjioglu, Asil; Ong, Eng Kok (University of Melbourne, Australia). PCT Int. Appl. WO 9304174 A1 19930304, 121 pp. DESIGNATED STATES: W: AU, CA, DK, JP, KR; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-AU430 19920814. PRIORITY: US 1991-746702 19910816.

AB Nucleic acid sequences are disclosed which code for 2 ryegrass pollen allergen Lol p Ib family members, as are purified Lol p Ib.1 and Lol p Ib.2 proteins and fragments thereof, methods for producing the recombinant Lol p Ib.1 and Lol p Ib.2 or fragments, derivs., or homologs thereof, and methods of using the nucleic acids and proteins. Cloning of allergen genes is described, and **nucleotide** sequences (and corresponding amino acid sequences) for nucleic acids coding Lol p Ib.1 and Lol p Ib.2 are included. Allergen epitopes were delineated with IgE and monoclonal antibodies (MAbs). Prodn. of MAbs against Lol p Ib.1 is also described. RNA was extd. from Lolium perenne flowerheads, and PCR was used in anal. of polymorphism of the genes encoding Lol p Ib.1 and Lol p Ib.2.

=> s polynucleotide
L3 36449 POLYNUCLEOTIDE

=> s l3 and allergen
L4 28 L3 AND ALLERGEN

=> s l4 and modified
L5 1 L4 AND MODIFIED

=> d l5 cbib abs

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS

2001:762863 Document No. 135:317456 Synergistic improvements to **polynucleotide** vaccines. Raz, Eyal; Takabayashi, Kenji; Nguyen, Minh-Duc (The Regents of the University of California, USA). PCT Int. Appl. WO 2001076642 A1 20011018, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US11290 20010406. PRIORITY: US 2000-PV195890 20000407.

AB The invention features a **polynucleotide** vaccine **modified** to enhance expression of the encoded antigen in host cells. The **polynucleotide** vaccine comprises an antigen-encoding nucleic acid sequence derived from a non-host species of a first phylum or first kingdom, wherein the native signal sequence of the antigen coding sequence is deleted and, optionally, replaced with a signal sequence of a polypeptide of a second phylum or a second kingdom that is functional in the host to be immunized (e.g., a viral signal sequence with a plant antigen-encoding sequence). In one embodiment, the signal sequence is a hemagglutinin A (HA) signal sequence, and the antigen is an **allergen** (e.g., plant **allergen**) or from a pathogen (e.g., a bacterium, virus or parasite). The **polynucleotide** vaccine of the invention provides a synergistic effect with an immunostimulatory sequence (ISS) adjuvant to not only maintain, but to enhance, the immune response to the encoded antigen.

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its structure diagram
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structure diagram, plus NTE and SEQ fields
FHITSTR ----- First HIT RN, its text modification, its CA index name, and
its structure diagram
FHITSEQ ----- First HIT RN, its text modification, its CA index name, its
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L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
AN 2001:762863 CAPLUS
DN 135:317456
TI Synergistic improvements to **polynucleotide** vaccines

IN Raz, Eyal; Takabayashi, Kenji; Nguyen, Minh-Duc
PA The Regents of the University of California, USA
SO PCT Int. Appl., 64 pp.
    CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001076642	A1	20011018	WO 2001-US11290	20010406

        W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
            CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
            HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
            LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
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        RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
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L1   2 S NUCLEOTIDE AND MODIFIED ALLERGEN
L2   2 DUP REMOVE L1 (0 DUPLICATES REMOVED)
L3   36449 S POLYNUCLEOTIDE
L4   28 S L3 AND ALLERGEN
L5   1 S L4 AND MODIFIED

=> dup remove l4
PROCESSING COMPLETED FOR L4
L6   28 DUP REMOVE L4 (0 DUPLICATES REMOVED)

=> d l6 1-28 cbib abs

L6 ANSWER 1 OF 28 CAPLUS COPYRIGHT 2002 ACS
2002:51938 Human uteroglobin-like **polynucleotides** and polypeptides
     and their recombinant production and uses. Ni, Jian; Ruben, Steven M.
     (USA). U.S. Pat. Appl. Publ. US 20020006640 A1 20020117, 113 pp.,
     Cont.-in-part of Appl. No. PCT/US00/30326. (English). CODEN: USXXCO.
     APPLICATION: US 2001-846258 20010502. PRIORITY: US 1999-PV163395
     19991104; WO 2000-US30326 20001103.
AB The present invention relates to two novel human cDNA mols. encoding
     proteins belonging to the uteroglobin family of proteins. Both
     uteroglobin-like proteins share sequence homol. with domestic cat major
     allergen I chain 1 (fel DI). One gene is expressed in the brain,
     testis, and ovarian cancer tissue and in resting T cells, and the second
     gene is expressed in testes and ovarian cancer tissue and resting T cells.
     Also provided are vectors, host cells, antibodies, and recombinant methods
     for producing human uteroglobin-like polypeptides. The invention further
     relates to diagnostic and therapeutic methods useful for diagnosing and
     treating disorders related to these novel human uteroglobin-like
     polypeptides.

L6 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2002 ACS
2001:935777 Document No. 136:65255 Plant gene promoters for the modification
     of gene expression. Perera, Ranjan; Rice, Stephen; Eagleton, Clare;

Lasham, Annette (Genesis Research + Development Corporation Limited, N. Z.; Fletcher Challenge Forests Industries Limited). PCT Int. Appl. WO 2001098485 A1 20011227, 121 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-NZ115 20010620. PRIORITY: US 2000-598401 20000620; US 2000-724624 20001128.

AB Novel isolated plant **polynucleotide** promoter sequences from Pinus radiata and Eucalyptus grandis are provided, together with genetic constructs comprising such **polynucleotides**. Methods for using such constructs in modulating the transcription of DNA sequences of interest are also disclosed, together with transgenic plants comprising such constructs. The regulatory DNA regions include constitutive promoters (i.e., from the Super ubiquitin gene), tissue-specific promoters (i.e., specific for leaf, root, flower, pollen, bud, and meristem expression), and temporally regulated promoters (i.e., for xylogenesis).

L6 ANSWER 3 OF 28 CAPLUS COPYRIGHT 2002 ACS

2001:923841 Document No. 136:49344 Codon optimized recombinant Dermatophagoides **allergen** ProDer p I, recombinant production, purification and IgE and IgG-reactivities. Bollen, Alex; Jacobs, Paul; Jacquet, Alain; Massaer, Marc Georges Francis (Smithkline Beecham Biologicals S.A., Belg.). PCT Int. Appl. WO 2001096385 A1 20011220, 48 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-EP6483 20010607. PRIORITY: GB 2000-14288 20000610.

AB The present invention relates to codon optimized **polynucleotides** which are efficiently expressed in mammalian cells and encode insect proteins from Dermatophagoides dust mite. In particular, the optimized codon **polynucleotides** encode a protein from Dermatophagoides pteronyssinus, such as Der P I or proDer P I. The codons are selected from highly expressed human gene and humanized ProDer p I gene was generated. The native Der p I signal sequence was exchanged with the highly efficient leader peptide of VZV glycoprotein E (gE) to facilitate secretion. The present invention also provides methods of prepg. pharmaceutical compns. comprising the expression of the codon optimized **polynucleotides**, and vectors and transformed host cells comprising them. The invention demonstrated that recombinant ProDer P I display reactivities similar to those of native Der P I towards specific anti-Der P I IgG and anti-Dermatophagoides pteronyssinus IgE, suggesting that recProDer P I displayed the overall structure of the natural **allergen**. The invention provides a method for efficient expression of recombinant Dermatophagoides **allergens** for use in the manuf. of pharmaceuticals, vaccine or diagnostic assays.

L6 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2002 ACS

2001:798084 Document No. 135:348865 Compositions and methods for in vivo delivery of **polynucleotide**-based therapeutics. Hartikka, Jukka; Sukhu, Loretta; Manthorpe, Marston (Vical Incorporated, USA). PCT Int. Appl. WO 2001080897 A2 20011101, 176 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US12975 20010423. PRIORITY: US 2000-PV198823 20000421; US 2000-PV253153 20001128.

AB The present invention relates to pharmaceutical compns. and methods to improve expression of exogenous polypeptides into vertebrate cells in vivo, utilizing delivery of **polynucleotides** encoding such polypeptides. More particularly, the present invention provides the use of salts, in particular sodium and potassium salts of phosphate, in aq. soln., and auxiliary agents, in particular detergents and surfactants, in pharmaceutical compns. and methods useful for direct **polynucleotide**-based polypeptide delivery into the cells of vertebrates.

L6 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2002 ACS
2001:780957 Document No. 135:343275 Sheep lice, Bovicola ovis, **allergen** treatment. Pfeffer, Alexander Terrence; Shoemaker, Charles Bix (Agresearch Limited, N. Z.). PCT Int. Appl. WO 2001079281 A1 20011025, 59 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-NZ65 20010419. PRIORITY: NZ 2000-504096 20000419.
AB The present invention concerns novel nucleotide sequences encoding a louse **allergen**, particularly although by no means exclusively from the chewing louse Bovicola ovis, and the use of said nucleotide sequences and protein **allergen** in the diagnosis, treatment and prevention of lice infestation and assocd. allergic disease.

L6 ANSWER 6 OF 28 CAPLUS COPYRIGHT 2002 ACS
2001:762863 Document No. 135:317456 Synergistic improvements to **polynucleotide** vaccines. Raz, Eyal; Takabayashi, Kenji; Nguyen, Minh-Duc (The Regents of the University of California, USA). PCT Int. Appl. WO 2001076642 A1 20011018, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US11290 20010406. PRIORITY: US 2000-PV195890 20000407.
AB The invention features a **polynucleotide** vaccine modified to enhance expression of the encoded antigen in host cells. The **polynucleotide** vaccine comprises an antigen-encoding nucleic acid sequence derived from a non-host species of a first phylum or first kingdom, wherein the native signal sequence of the antigen coding sequence is deleted and, optionally, replaced with a signal sequence of a polypeptide of a second phylum or a second kingdom that is functional in the host to be immunized (e.g., a viral signal sequence with a plant antigen-encoding sequence). In one embodiment, the signal sequence is a hemagglutinin A (HA) signal sequence, and the antigen is an **allergen** (e.g., plant **allergen**) or from a pathogen (e.g., a bacterium, virus or parasite). The **polynucleotide** vaccine of the invention provides a synergistic effect with an immunostimulatory sequence (ISS) adjuvant to not only maintain, but to enhance, the immune response to the encoded antigen.

L6 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2002 ACS
2001:747853 Document No. 135:302897 CD20/IgE-receptor like molecules and uses thereof. Welcher, Andrew A.; Calzone, Frank J. (Amgen, Inc., USA). PCT Int. Appl. WO 2001074903 A2 20011011, 145 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU,

CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US10048 20010329. PRIORITY: US 2000-PV193728 20000330; US 2000-723258 20001127.

AB Novel CD20/IgE-receptor like polypeptides and nucleic acid mols. encoding the same. The invention also provides vectors, host cells, agonists and antagonists (including selective binding agents), and methods for producing CD20/IgE-receptor like polypeptides. Also provided for are methods for the treatment, diagnosis, amelioration, or prevention of diseases with CD20/IgE-receptor like polypeptides.

L6 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2002 ACS

2001:566874 Document No. 135:163416 Methods and materials relating to human hematopoietic cytokine-like polypeptides and **polynucleotides**. Boyle, Bryan J.; Mize, Nancy K.; Arterburn, Matthew C.; Palencia, Servando; Tang, Y. Tom; Liu, Chenghua; Drmanac, Rodje T. (Hyseq, Inc., USA). PCT Int. Appl. WO 2001055435 A2 20010802, 150 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US2612 20010125. PRIORITY: US 2000-491404 20000125; US 2000-491040 20000125; US 2000-684147 20001005.

AB The invention provides **polynucleotides** and polypeptides encoded by such **polynucleotides** and mutants or variants thereof that correspond to a novel human secreted hematopoietic cytokine (HC) -like polypeptide. These **polynucleotides** (Hyseq clone identification nos. 15097076 (SEQ ID NO:1)) comprise nucleic acid sequences isolated from a human thymus cDNA library. This novel HC-like polypeptide also has sequence homol. to human prostate cancer overexpressed gene 1 protein (SEQ ID NO:12) and human liver cell clone HP10301 (SEQ ID NO:13). A sequence variant of the novel HC-like polypeptide with four amino acid changes was isolated from a human placenta cDNA library. The novel HC-like polypeptide has a predicted signal sequence, an erythropoietin/thrombopoietin domain, a bacterial opsin domain, and a pollen **allergen** amb family domain. Other aspects of the invention include vectors contg. processes for producing novel human secreted HC-like polypeptides, and antibodies specific for such polypeptides. The **polynucleotides** and polypeptides of this invention may be used for diagnosis, drug screening, and therapy.

L6 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2002 ACS

2001:525947 Document No. 135:112005 Composition of antigen and glycolipid adjuvant sublingual administration. Wheeler, Alan; Elliott, Garry; Cluff, Christopher Wallace (Allergy Therapeutics Limited, UK; Corixa Corporation). PCT Int. Appl. WO 2001051082 A1 20010719, 55 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-GB142 20010115. PRIORITY: GB 2000-891 20000114.

AB A method of producing a mucosal and systemic immune response in a mammal

comprising administering sublingually an effective amt. of a compn. comprising at least one antigen and a glycolipid adjuvant to said mammal. An example is given for prepn. of MPL (a form of 3 de-O-acylated monophosphoryl lipid A) adjuvant with DPPC.

L6 ANSWER 10 OF 28 CAPLUS COPYRIGHT 2002 ACS
2001:380427 Document No. 135:496 Immunomodulatory compositions containing an immunostimulatory sequence linked to antigen and methods of use thereof. Tuck, Stephen; Van Nest, Gary (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2001035991 A2 20010525, 97 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31385 20001115. PRIORITY: US 1999-PV165467 19991115; US 2000-713136 20001114.
AB The invention provides classes of immunomodulatory compns. which comprise an av. of one or more immunostimulatory sequence (ISS) contg. **polynucleotide** conjugated, or attached, to antigen. The extent of conjugation affects immunomodulatory properties, such as extent of antigen-specific antibody formation, including Th1-assocd. antibody formation, and thus these various conjugate classes are useful for modulating the type and extent of immune response. The invention also includes methods of modulating an immune response using these compns.

L6 ANSWER 11 OF 28 CAPLUS COPYRIGHT 2002 ACS
2001:137053 Document No. 134:192226 Methods of modulating an immune response using immunostimulatory sequences and compositions for use therein. Van Nest, Gary (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2001012223 A2 20010222, 63 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US22835 20000818. PRIORITY: US 1999-PV149768 19990819.
AB The invention provides methods of modulating an immune response to a second antigen which entail administration of a first antigen and an immunostimulatory **polynucleotide**. Modulation of the immune response is generally manifested as stimulation of a Th1 response.

L6 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2002 ACS
2000:911095 Document No. 134:70358 Chimeric chemokine-antigen polypeptides and uses therefor. Garzino-Demo, Alfredo; Gallo, Robert C.; Lim, Siew Pheng; Tan, Yin Hwee (University of Maryland Biotechnology Institute, USA; Institute of Molecular and Cell Biology). PCT Int. Appl. WO 2000078334 A1 20001228, 127 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US16598 20000616. PRIORITY: US 1999-335150 19990617.
AB The present invention provides chimeric polypeptides comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following

groups: fragments of chemokines, analogs of chemokines, derivs. of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one ore more polypeptide linkers connecting the one ore more chemokine polypeptides to the one or more antigenic polypeptide(s), and methods of making such chimeric polypeptides and methods of using such polypeptides for eliciting or enhancing an immune response. The chemokine is selected from C, CXC, C-C, and CX3C; antigen is selected from autoantigen, **allergen**, tumor-assocd. antigen or antigens derived from plant, fungi, protozoa, bacteria and virus; and the linker polypeptide is an antibody hinge region derived from heavy chain of IgG2a or IgG2b.

L6 ANSWER 13 OF 28 CAPLUS COPYRIGHT 2002 ACS
2000:707298 Document No. 133:277189 Forestry plant gene promoters for the modification of gene expression. Perera, Ranjan; Rice, Stephen J.; Eagleton, Clare Katherine (Genesis Research & Development Corporation Ltd., N. Z.; Fletcher Challenge Forests Ltd.). PCT Int. Appl. WO 2000058474 A1 20001005, 93 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-NZ18 20000224. PRIORITY: US 1999-276599 19990325; US 1999-PV146591 19990730.

AB Novel isolated plant **polynucleotide** promoter sequences from Pinus radiata and Eucalyptus grandis are provided, together with DNA constructs comprising such **polynucleotides**. The regulatory DNA regions include constitutive promoters (i.e., from the Super ubiquitin gene), tissue-specific promoters (i.e., specific for leaf, root, flower, pollen, bud, and meristem expression), and temporally regulated promoters (i.e., for xylogenesis). Methods for using such constructs in modulating the transcription of DNA sequences of interest are also disclosed, together with transgenic plants comprising such constructs.

L6 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2002 ACS
2000:707018 Document No. 133:280556 Adjuvant compositions and methods for enhancing immune responses to **polynucleotide**-based vaccines. Wheeler, Carl J. (Vical Incorporated, USA). PCT Int. Appl. WO 2000057917 A2 20001005, 72 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US8282 20000324. PRIORITY: US 1999-PV126340 19990326.

AB The invention provides adjuvants, immunogenic compns., and methods useful for **polynucleotide**-based vaccination and immune response. In particular, the invention provides an adjuvant of cytofectin:co-lipid mixt. wherein cytofectin is GAP-DMORIE.

L6 ANSWER 15 OF 28 CAPLUS COPYRIGHT 2002 ACS
2000:384227 Document No. 133:29600 Capsid particles of hepatitis B core antigen for presentation of immunogenic components. Murray, Kenneth (Biogen, Inc., USA). PCT Int. Appl. WO 2000032625 A1 20000608, 60 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US28755 19991203. PRIORITY: US 1998-PV110911 19981204.

AB The authors discloses the use of hepatitis B virus (HBV) core antigen

particles for presentation to the immune system of multiple immunogen specificities. The immunogens, epitopes, or other related structures, are crosslinked or fused to HBV capsid-binding peptides that selectively bind to HBV core protein. Mixts. of different immunogens and/or capsid-binding peptide ligands may be crosslinked to the same HBV core particle. Such resulting multicomponent or multivalent HBV core particles may be advantageously used in therapeutic and prophylactic vaccines and compns., as well as in diagnostic applications.

L6 ANSWER 16 OF 28 CAPLUS COPYRIGHT 2002 ACS
2000:209943 Document No. 132:246356 Methods and compositions using an IgE inhibitor and an antigen and/or immunostimulatory **polynucleotide** for treating IgE-associated disorders. Dina, Dino (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2000016804 A1 20000330, 42 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US21686 19990917. PRIORITY: US 1998-100838 19980918; US 1999-136600 19990528; US 1999-397198 19990916.
AB The invention provides methods of treating IgE-assocd. disorders and compns. for use therein. The methods are particularly useful in treatment of allergies and allergy-related disorders. The methods generally comprise administering an IgE inhibitor (such as anti-IgE antibody) and an antigen and/or immunostimulatory **polynucleotide** sequence (ISS). These combination methods offer significant advantages, such as allowing more aggressive therapy while reducing unwanted side effects, such as anaphylaxis.

L6 ANSWER 17 OF 28 CAPLUS COPYRIGHT 2002 ACS
2000:144744 Document No. 132:177741 Method for detection of biological factors in epidermis. Rheins, Lawrence A.; Morhenn, Vera B. (California Skin Research Institute, USA). PCT Int. Appl. WO 2000010579 A1 20000302, 34 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US19012 19990817. PRIORITY: US 1998-97025 19980818.
AB Disclosed is a method for removing **polynucleotide** from the skin. This **polynucleotide** can be used to detect dermatitis and distinguish an irritant reaction from an allergic reaction by characterizing the **polynucleotide** according to the polypeptide which it encodes. Addnl., provided are methods for non-invasive isolation of samples from the skin as well as kits for use in the methods provided herein.

L6 ANSWER 18 OF 28 CAPLUS COPYRIGHT 2002 ACS
2000:104519 Document No. 132:165114 Compound and method for the prevention and/or the treatment of allergy. Saint-Remy, Jean-Marie; Jacquemin, Marc (UCB S. A., Belg.). PCT Int. Appl. WO 2000006694 A2 20000210, 50 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN:

PIXXD2. APPLICATION: WO 1999-BE92 19990720. PRIORITY: EP 1998-870167 19980730.

AB The present invention is related to a compd. for the prevention and/or the treatment of allergy consisting of: at least one **allergen** antigenic determinant which is recognized by a B cell or an antibody secreted by a B cell of a non-atopic individual to said **allergen**, and at least one antigenic determinant of an antigen different from said **allergen** which triggers T cell activation. Thus, peptides or proteins contg. T cell epitope of tetanus toxoid and/or B cell epitope of Der p II **allergen**, or polypeptide contg. T cell epitope of influenza A virus and B cell epitope of Der p I **allergen** were prepd. for administration by gene transfer technol. through adenoviral vehicle, or by oral through food (e.g. acidified whey milk).

L6 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2002 ACS

1999:736928 Document No. 131:350264 Antibodies to dendritic cells and human dendritic cell populations with therapeutic and diagnostic applications involving vaccines and adoptive immunotherapy. Rieber, Ernst Peter (Micromet G.m.b.H., Germany). PCT Int. Appl. WO 9958678 A2 19991118, 121 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-EP3218 19990511. PRIORITY: EP 1998-108534 19980511.

AB Antibodies specifically recognizing a distinct population of human dendritic cells (DCs) and methods of isolating said DCs using said antibodies. Furthermore, antigens and epitopes recognized by the above-described antibodies as well as **polynucleotides** encoding said antibodies. Also vectors comprising said **polynucleotides** as well as host cells transformed therewith and their use in the prodn. of said antibodies. Addnl., polypeptides comprising a domain of the binding site of the aforementioned antibodies, or an antigen or epitope described above and at least one further, preferably functional domain as well as **polynucleotides** encoding such polypeptides. Furthermore, vectors comprising said **polynucleotides**, host cells transfected with said **polynucleotide** or vector and their use for the prepn. of the above-described polypeptides. Further a method for isolating or identifying DCs as defined above as well as DCs obtainable by said method and/or characterized by recognition of the above-described antibody, and/or contg. the aforementioned antigen or epitope. Moreover, a method for prepg. or identifying T cells in a certain status as well as methods for identifying compds. which interfere with T cell mediated activation of immune responses. In addn. kits, and compns., preferably pharmaceutical and diagnostic compns. are provided comprising any of the afore described antibodies, antigens, epitopes, polypeptides, **polynucleotides**, vectors, dendritic cells or T cells or compds. obtainable by the aforementioned method. Methods for identifying mols. synthesized by dendritic cells having enhancing or modulating or suppressing effects on antigen-activation of T cells by gene expression comparisons. Method for dendrite cell propagation are described assocd. with growth in cytokine cocktail and cell immortalization. Activation of T cells against recall and neoantigens is also described.

L6 ANSWER 20 OF 28 CAPLUS COPYRIGHT 2002 ACS

1999:529035 Document No. 131:165306 Specific inhibitors of NFAT activation by calcineurin, use in treating immune-related diseases, and screening methods. Hogan, Patrick G.; Rao, Anjana; Aramburu, Jose (Center for Blood Research, Inc., USA). PCT Int. Appl. WO 9940930 A1 19990819, 125 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION:

WO 1999-US3085 19990211. PRIORITY: US 1998-PV74467 19980212.

AB Isolated peptide fragments of the conserved regulatory domain of NFAT protein capable of inhibiting protein-protein interaction between calcineurin and NFAT, or a biol. active analog thereof, are described. Isolated **polynucleotides** and gene therapy vectors encoding the peptide fragments are also described. In addn., methods for treating immune-related diseases or conditions and methods for high throughput screening of candidate agents are described. Pharmaceutical compns. are also provided.

L6 ANSWER 21 OF 28 CAPLUS COPYRIGHT 2002 ACS

1998:604845 Document No. 129:232434 Separator for continuous flow isoelectric focusing for purifying biological substances. De Boer, Gerben Foppe; Sova, Oto (Cerberus Developments B.V., Neth.). PCT Int. Appl. WO 9836821 A1 19980827, 41 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-NL104 19980220.

AB Charged biol. substances are recovered from solns. or suspensions by isoelec. focusing in a continuous flow separator at flow rates of 25-250 L/L-day. The app. comprises a sepg. chamber, a pair of vertical electrodes located at the sides of the chamber, an anion-selective or a cation-selective membrane located near each of the electrodes for sepg. electrode spaces from a central part of the app., one or more outflow ports for sepg. liq. fractions at the upper part of the chamber, two secondary outflow ports in the upper part of each of the electrode spaces, one or more vertical wall(s) between each of the outflow ports partitioning the chamber at the height of the outflow ports, and a plurality of vertical permeable partition walls to enable a convection-free upward flow. The app. does not require addnl. amphoteric buffering solns. to establish a suitable pH gradient, and can be used for purifn. of **polynucleotides**, amino acids, plasmids, peptides, proteins, enzymes, Igs, antigens, org. solvents and beverages.

L6 ANSWER 22 OF 28 CAPLUS COPYRIGHT 2002 ACS

1998:493693 Document No. 129:121651 Compounds, compositions and methods for the endocytic presentation of immunosuppressive factors. Zaghouani, Habib (Alliance Pharmaceutical Corp., USA; Zaghouani, Habib). PCT Int. Appl. WO 9830706 A1 19980716, 71 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US520 19980107. PRIORITY: US 1997-779767 19970107.

AB Immunomodulating agents comprising at least one Fc receptor ligand and at least one immunosuppressive factor are provided as are methods for their manuf. and use. The immunomodulating agents may be in the form of polypeptides or chimeric antibodies and preferably incorporate an immunosuppressive factor comprising a T cell receptor antagonist or agonist. The compds. and compns. of the invention may be used to selectively suppress the immune system to treat symptoms assocd. with immune disorders such as allergies, transplanted tissue rejection and autoimmune disorders including lupus, rheumatoid arthritis and multiple sclerosis.

L6 ANSWER 23 OF 28 CAPLUS COPYRIGHT 2002 ACS

1998:388603 Document No. 129:40131 Vaccines for inducing cell-mediated

cytolytic response comprising antigen and stress protein. Mizzen, Lee; Anthony, Lawrence S. D. (Stressgen Biotechnologies Corp., Can.; Mizzen, Lee; Anthony, Lawrence S. D.). PCT Int. Appl. WO 9823735 A1 19980604, 71 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-CA897 19971125. PRIORITY: US 1996-756621 19961126.

AB The present invention relates to a vaccine for inducing an immune response to an antigen in a vertebrate (e.g., mammal) comprising an antigen and all or a portion of a stress protein or all or a portion of a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of the stress protein to induce the immune response against the antigen. In a particular embodiment, the present invention relates to vaccines and compns. which induce a CTL response in a mammal comprising an antigen and all or a portion of a stress protein. In another embodiment, the invention relates to vaccines and compns. which induce an immune response to an influenza virus in a mammal comprising an antigen of the influenza virus and all or a portion of one or more stress proteins. The invention also relates to vaccines and compns. for inducing a CTL response to a tumor-assocd. antigen comprising a tumor-assocd. antigen and all or a portion of the stress protein. The invention also relates to vaccines and compn. for suppressing allergic immune responses to **allergens** comprising an **allergen** and all or a portion of a stress protein. Immunogens comprising influenza virus NP peptide and Mycobacterium hsp70, NP peptide-hsp70 conjugates and NP peptide-hsp70 fusion proteins were prepd. Mice immunized with these prepns. displayed a CTL response against cells exhibiting the NP peptide.

L6 ANSWER 24 OF 28 CAPLUS COPYRIGHT 2002 ACS

1998:602972 Document No. 129:215710 Methods and devices for immunizing a host through administration of naked **polynucleotides** with encode allergenic peptides. Carson, Dennis A.; Raz, Eyal; Howell, Meredith L. (The Regents of the University of California, USA). U.S. US 5804566 A 19980908, 39 pp., Cont.-in-part of U.S. Ser. No. 112,440, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1994-333068 19941101. PRIORITY: US 1993-112440 19930826.

AB The invention is directed to methods for introducing biol. active peptides into a host by administration of **polynucleotides** which operatively encode for the peptide of interest. In a preferred embodiment of the invention, a mammal is desensitized to an antigen, in particular an **allergen**, through administration to the mammal of **polynucleotides** which operatively encode the antigen. The antigen-encoding **polynucleotides** are administered to host tissues which have a high concn. of antigen presenting cells in them relative to other host tissues. The method is particularly useful in treating allergies because the **allergen**-encoding **polynucleotides** of the invention to induce tolerance while suppressing IgE antibody formation. Devices and compns. for use in the methods of the invention are also described. Thus, naked cDNA encoding rearranged .kappa. light gene of autoantibody IgM derived from human patient with chronic lymphocytic leukemia and plasmid encoding influenza ribonucleoprotein were prepd. and tested.

L6 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2002 ACS

1998:147629 Document No. 128:138317 Analytical device for biochemical analysis and diagnosis, including immunoassay, enzyme analysis, and detection of viruses. Legastelois, Stephane (Indicia Diagnostics, Fr.). Fr. Demande FR 2746924 A1 19971003, 22 pp. (French). CODEN: FRXXBL. APPLICATION: FR 1996-3987 19960329.

AB A device for detecting or detg. analytes in liq. samples consists of a

solid annular support closed by a polymeric film. The device is prepd. by immersing the annular support in a polymer soln. contg. appropriate reagents and the liq. film is allowed to solidify by evapn. or cross linking. Reagents may also be immobilized after formation of the film. For anal. purposes, the film is immersed in test fluids and the appropriate analyte is detected or detd. by established procedures. Applications include the detection or detn. of antigens, antibodies, hormones, or enzymes. Thus, serum creatine kinase is detd. by using hexokinase, glucose 6-phosphate dehydrogenase, and appropriate substrates in gelatin and the prodn. of NADPH is quantified by UV spectrometry.

L6 ANSWER 26 OF 28 CAPLUS COPYRIGHT 2002 ACS

1997:165250 Document No. 126:154826 Functional surrogates of analytes of interest and methods of obtaining and using same. Lee-Own, F. Victor; Carter, John Mark (Cytogen Corporation, USA). PCT Int. Appl. WO 9641172 A1 19961219, 154 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US10498 19960607. PRIORITY: US 1995-476375 19950607.

AB Functional surrogates are disclosed which serve as mimics of naturally occurring mols., such as analytes of interest present in a given sample. In particular, functional surrogates (including epitopes and mimetopes) of macromol. moieties, including large to medium-sized proteins, are described. The functional surrogates of the present invention are useful in a variety of diagnostic, prophylactic, and therapeutic applications. Indeed, where the detection of a macromol. moiety is hampered by its size, a functional surrogate of the present invention, serving as the mimic of the macromol. moiety, may be better suited for a given diagnostic assay. Methods of obtaining functional surrogates, various methods of their use, and compns., including kits, are also described. Accordingly, certain binding peptides, including those of a well-defined sequence, have been discovered, which can be used in a no. of affinity assays, including those utilizing fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), or cloned enzyme donor immunoassays (CEDIA), to name a few.

L6 ANSWER 27 OF 28 CAPLUS COPYRIGHT 2002 ACS

1996:443985 Document No. 125:96036 Methods and devices for immunizing a host through administration of naked **polynucleotides** which encode antigenic peptides. Carson, Dennis A.; Raz, Eyal; Howell, Meredith L. (Regents of the University of California, USA). PCT Int. Appl. WO 9613277 A1 19960509, 97 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US14203 19951031. PRIORITY: US 1994-333068 19941101.

AB The invention is directed to methods for introducing biol. active peptides into a host by administration of **polynucleotides** which operatively encode for the peptide of interest. In a preferred embodiment of the invention, a mammal is desensitized to an antigen, in particular an **allergen**, through administration to the mammal of **polynucleotides** which operatively encode the antigen. The antigen-encoding **polynucleotides** are administered to host tissues which have a high concn. of antigen presenting cells in them relative to other host tissues. The method is particularly useful in treating allergies because the **allergen**-encoding **polynucleotides** of the invention induce tolerance while suppressing IgE antibody formation. Entodermic inoculation needle devices and compns. for use in the methods of the invention are also described.

L6 ANSWER 28 OF 28 CAPLUS COPYRIGHT 2002 ACS
1992:569421 Document No. 117:169421 Birch pollen **allergen** P14 for diagnosis and therapy of allergic diseases, and recombinant production of the **allergen**. Valenta, Rudolf; Duchene, Michael; Pettenburger, Karin; Breitenbach, Michael; Kraft, Dietrich; Rumpold, Helmut; Scheiner, Otto (Biomay Biotechnik Produktions- und Handelsgesellschaft m.b.H., Austria). PCT Int. Appl. WO 9203551 A1 19920305, 69 pp. DESIGNATED STATES: W: AU, CA, FI, JP, NO, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-EP1513 19910809. PRIORITY: AT 1990-1685 19900813; US 1991-683832 19910411.

AB Recombinant DNAs are provided which code for polypeptides having the antigenicity of P14 **allergen** of birch (Betula verrucosa) and other plants of the order Fagales (and for polypeptides comprising .gtoreq.1 epitope thereof). Methods of producing the proteins and polypeptides are disclosed, as is their use in diagnosis and therapy of allergic diseases. A method for purifn. of P14 **allergens** or cross-reactive **allergens** using binding to poly(L-proline) is also disclosed. The **polynucleotide** coding for birch P14 was inserted in plasmid pKK223-3 for prodn. of a recombinant nonfusion protein, while a recombinant fusion protein was produced using plasmid pEXB. Reactivity of the produced polypeptides with patient IgE is shown. Homol. of the birch P14 sequence with a variety of profilin sequences is included.

=> s allergen
L7 97315 ALLERGEN

=> s l7 and modified
L8 2206 L7 AND MODIFIED

=> s l8 and polynucleotide
L9 1 L8 AND POLYNUCLEOTIDE

=> d l9 cbib abs

L9 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2001:762863 Document No. 135:317456 Synergistic improvements to **polynucleotide** vaccines. Raz, Eyal; Takabayashi, Kenji; Nguyen, Minh-Duc (The Regents of the University of California, USA). PCT Int. Appl. WO 2001076642 A1 20011018, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US11290 20010406. PRIORITY: US 2000-PV195890 20000407.

AB The invention features a **polynucleotide** vaccine **modified** to enhance expression of the encoded antigen in host cells. The **polynucleotide** vaccine comprises an antigen-encoding nucleic acid sequence derived from a non-host species of a first phylum or first kingdom, wherein the native signal sequence of the antigen coding sequence is deleted and, optionally, replaced with a signal sequence of a polypeptide of a second phylum or a second kingdom that is functional in the host to be immunized (e.g., a viral signal sequence with a plant antigen-encoding sequence). In one embodiment, the signal sequence is a hemagglutinin A (HA) signal sequence, and the antigen is an **allergen** (e.g., plant **allergen**) or from a pathogen (e.g., a bacterium, virus or parasite). The **polynucleotide**

vaccine of the invention provides a synergistic effect with an immunostimulatory sequence (ISS) adjuvant to not only maintain, but to enhance, the immune response to the encoded antigen.

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=> s peanut allergen
L10        469 PEANUT ALLERGEN

=> s l10 and polynucleotide
L11          0 L10 AND POLYNUCLEOTIDE

=> s l10 and reduce IgE binding
L12          0 L10 AND REDUCE IGE BINDING

=> s l10 and Ara h1
L13         15 L10 AND ARA H1

=> s l13 and polynucleotide
L14          0 L13 AND POLYNUCLEOTIDE

=> dup remove l13
PROCESSING COMPLETED FOR L13
L15           8 DUP REMOVE L13 (7 DUPLICATES REMOVED)

=> d l15 1-8 cbib abs

L15  ANSWER 1 OF 8  CAPLUS  COPYRIGHT 2002 ACS
2001:380762  Document No. 135:1229  Isolation and sequence of a full length
     genomic clone for allergen Ara h2 and down-regulation and silencing of
     allergen genes in transgenic peanut seeds.  Dodo, Hortense W.; Arntzen,
     Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University,
     USA).  PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp.  DESIGNATED
     STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
     CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
     ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
     MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
     SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG,
     KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK,
     ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG, TR.  (English).  CODEN: PIXXD2.  APPLICATION: WO 2000-US31657
     20001120.  PRIORITY: US 1999-PV167255 19991119.
AB   An allergen-free transgenic peanut seed is produced by recombinant
     methods.  Peanut plants are transformed with multiple copies of each of
     the allergen genes, or fragments thereof, to suppress gene expression and
     allergen protein prodn.  Alternatively, peanut plants are transformed with
     peanut allergen antisense genes introduced into the
     peanut genome as antisense fragments, sense fragments, or combinations of
     both antisense and sense fragments.  Peanut transgenes are under the
     control of the 35S promoter, or the promoter of the Ara h2 gene to produce
     antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing
     allergen protein prodn. in peanut plants.  A full length genomic clone for
     allergen Ara h2 is isolated and sequenced.  The ORF is 622 nucleotides
     long.  The predicted encoded protein is 207 amino acids long and includes
     a putative transit peptide of 21 residues.  One polyadenilation signal is
     identified at position 951.  Six addnl. stop codons are obsd.  A promoter
     region was revealed contg. a putative TATA box located at position -72.
     Homologous regions were identified between Ara h2, h6, and h7, and between
     Ara h3 and h4, and between Ara h1P41B and Ara h1P17.  The homologous
     regions will be used for the screening of peanut genomic library to
     isolate all peanut allergen genes and for
     down-regulation and silencing of multiple peanut
     allergen genes.

L15  ANSWER 2 OF 8  CAPLUS  COPYRIGHT 2002 ACS
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2001:295491 Document No. 135:151859 Detection of **peanut allergens** in breast milk of lactating women. Vadas, Peter; Wai, Yvonne; Burks, Wesley; Perelman, Boris (Division of Allergy and Clinical Immunology, St Michael's Hospital, University of Toronto, Toronto, ON, Can.). JAMA, J. Am. Med. Assoc., 285(13), 1746-1748 (English) 2001. CODEN: JAMAAP. ISSN: 0098-7484. Publisher: American Medical Association.

AB Most individuals who react to peanuts do so on their 1st known exposure. A potential but unproven route of occult exposure resulting in sensitization to peanut is via breast milk during lactation. To investigate the ability of maternal dietary peanut protein to pass into breast milk during lactation. Clin. investigation conducted at 2 North American hospitals from Mar. 1999 to Oct. 2000 including 23 healthy, lactating women aged 21-35 yr. Each woman consumed 50 g of dry roasted peanuts, after which breast milk samples were collected at hourly intervals. Presence in breast milk of total peanut protein, analyzed by a sandwich ELISA, and 2 major **peanut allergens**, Ara h 1 and Ara h 2, detected by immunoblot anal. Peanut protein was detected in 11 of 23 subjects. It was detected in 10 subjects within 2 h of ingestion and in 1 subject within 6 h. The median peak peanut protein concn. in breast milk was 200 ng/mL (mean, 222 ng/mL; range, 120-430 ng/mL). Both major **peanut allergens** Ara h 1 and Ara h 2 were detected. Conclusions Peanut protein is secreted into breast milk of lactating women following maternal dietary ingestion. Exposure to peanut protein during breastfeeding is a route of occult exposure that may result in sensitization of at-risk infants.

L15 ANSWER 3 OF 8 MEDLINE DUPLICATE 1

2001435540 Document Number: 21227186. PubMed ID: 11328490. Hypogin, a novel antifungal peptide from peanuts with sequence similarity to **peanut allergen**. Ye X Y; Ng T B. (Department of Biochemistry, Faculty of Medicine, Chinese University of Hong Kong, Shatin, Hong Kong, China.) JOURNAL OF PEPTIDE RESEARCH, (2001 Apr) 57 (4) 330-6. Journal code: CTZ; 9707067. ISSN: 1397-002X. Pub. country: Denmark. Language: English.

AB A protein designated hypogin, with a prominent suppressive action on the growth of the fungi Mycosphaerella arachidicola, Fusarium oxysporum and Coprinus comatus, was isolated from seeds of the peanut Arachis hypogaea. The protein inhibited human immunodeficiency virus (HIV) reverse transcriptase and enzymes associated with HIV infection including alpha-glucosidase and beta-glucosidase. The proliferative response of mouse splenocytes was attenuated in the presence of the protein. The protein exhibited a molecular mass of 7.2 kDa in tricine gel electrophoresis and gel filtration on Superdex 75 and an N-terminal sequence resembling **peanut allergen Ara h1**. The isolation procedure involved affinity chromatography on Affi-gel blue gel and ion-exchange chromatography on CM-Sepharose. The protein was adsorbed in both chromatographic media.

L15 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2002 ACS

2001:408584 Document No. 135:45362 Peanut (Arachis hypogaea) - a common cause of food allergy. Wroblewska, Barbara; Jedrychowski, Lucjan (Inst. Rozrodu Zwierzat i Badan Zywnosci, Polska Akademia Nauk, Olsztyn, 10-747, Pol.). Zywnosc, 7(4), 104-113 (Polish) 2000. CODEN: ZYWNFL. Publisher: Polskie Towarzystwo Technologow Zywnosci, Oddzial Malopolski.

AB The Ridascreen Peanut sandwich ELISA test (R-Biopharm) assay kit was used to det. the main **peanut allergen Ara h1** content in 9 samples of raw plant material from peanuts (Arachis hypogaea), almonds (Amygdalus communis), soybean (Glycine max), oats, barley, wheat, buckwheat (Fagopyrum sagitatum), walnuts (Juglans regia), hazelnuts (Corylus avellana) and in 10 samples of sweet peanut confectionery (chocolate products, chocolate bars, sweet cream spreads). The assay cross-reactivity was examd. The **Ara h1** allergen was found in all sweet confectionery samples, even in those that were not labeled to contain peanuts. Three raw materials (almonds,

soybeans, oats) cross-reacted with the **Ara h1** antibody.

L15 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2002 ACS
1998:383080 Document No. 129:121594 Biochemical and structural analysis of the IgE binding sites on **Ara h1**, an abundant and highly allergenic peanut protein. Shin, David S.; Compadre, Cesar M.; Maleki, Soheila J.; Kopper, Randall A.; Sampson, Hugh; Huang, Shau K.; Burks, A. Wesley; Bannon, Gary A. (Department of Biochemistry & Molecular Biology, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA). J. Biol. Chem., 273(22), 13753-13759 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB Allergy to peanut is a significant IgE-mediated health problem because of the high prevalence, potential severity, and chronicity of the reaction. **Ara h1**, an abundant peanut protein, is recognized by serum IgE from >90% of peanut-sensitive individuals. It has been shown to belong to the vicilin family of seed storage proteins and to contain 23 linear IgE binding epitopes. Here, the authors detd. the crit. amino acids within each of the IgE binding epitopes of **Ara h1** that are important for Ig binding. Surprisingly, substitution of a single amino acid within each of the epitopes led to loss of IgE binding. In addn., hydrophobic residues appeared to be most crit. for IgE binding. The position of each of the IgE binding epitopes on a homol.-based mol. model of **Ara h1** showed that they were clustered into 2 main regions, despite their more even distribution in the primary sequence. Finally, the authors have shown that **Ara h1** forms a stable trimer by the use of a reproducible fluorescence assay. This information will be important in studies designed to reduce the risk of peanut-induced anaphylaxis by lowering the IgE binding capacity of the allergen.

L15 ANSWER 6 OF 8 MEDLINE DUPLICATE 2
1998339794 Document Number: 98339794. PubMed ID: 9677140. Identification and partial characterization of multiple major allergens in peanut proteins. de Jong E C; Van Zijverden M; Spanhaak S; Koppelman S J; Pellegrom H; Penninks A H. (TNO Nutrition and Food Research Institute, Immunotoxicology group, Zeist, The Netherlands.) CLINICAL AND EXPERIMENTAL ALLERGY, (1998 Jun) 28 (6) 743-51. Journal code: CEB; 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.
AB BACKGROUND: Peanuts are a major cause of food allergies both in children as in adults which can induce an anaphylactic shock. The identification and characterization of **peanut allergens** could lead to more insight into the mechanism and contribute to the improvement of diagnostic tests and treatment for peanut allergy. OBJECTIVE: In the present study, the peanut protein-specific immunoglobulin concentrations as well as their recognition of the various peanut proteins or protein subunits was determined in the plasma of peanut-allergic (PA) and non-allergic (NA) individuals. Moreover, two **peanut allergens** were characterized in more detail to confirm them as the earlier described **Ara h1** and Ara h2. METHODS: The presence of Ig-binding sites in peanut proteins was studied by immunoblotting assays whereas the concentrations of peanut-specific Ig was determined by ELISA. RESULTS: Peanut proteins were found to contain multiple binding sites for immunoglobulins. Of these proteins, six were recognized by peanut-specific IgE present in more than 50% of the plasma samples of the PA group. Their molecular weights were approximately 44, 40, 33, 21, 20 and 18 kDa. The last three protein bands were recognized by peanut-specific IgE present in more than 70% of the PA plasma samples and were thought to contain Ara h2. This allergen as well as another protein that was thought to be **Ara h1**, which was not recognized by the majority of the patients' IgE-containing plasma samples, were isolated and the N terminal amino acid sequence was determined.

Peanut protein-specific IgA, IgM, IgG and IgG-subclasses showed a more diverse recognition pattern of peanut protein in the PA group compared to the NA group. No differences were found in the plasma concentrations of peanut protein-specific immunoglobulins of the various classes between the PA and NA group. CONCLUSIONS: From the present study, we conclude that peanuts contain multiple allergens, of which six can be described as major allergens, Ara h2 included. In our population **Ara h1** is not a major allergen. The recognition of peanut proteins by immunoglobulins is more diverse in PA individuals compared with NA individuals which, however, is not substantiated in the concentrations of peanut-specific immunoglobulins in plasma, other than IgE.

L15 ANSWER 7 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:220385 Document No.: PREV199799512101. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. Burks, A. Wesley; Shin, David; Cockrell, Gael; Stanley, J. Steven; Helm, Ricki M.; Bannon, Gary A. (1). (1) Univ. Arkansas Med. Sci., Slot 516, 4301 W. Markham, Little Rock, AR 72205 USA. European Journal of Biochemistry, (1997) Vol. 245, No. 2, pp. 334-339. ISSN: 0014-2956. Language: English.

AB Peanut allergy is a significant health problem because of the prevalence and potential severity of the allergic reaction. Serum IgE from patients with documented peanut hypersensitivity reactions and overlapping peptides were used to identify the IgE-binding epitopes on the major **peanut allergen**, Ara h 1. At least twenty-three different linear IgE-binding epitopes, located throughout the length of the Ara h 1 protein, were identified. All of the epitopes were 6-10 amino acids in length, but there was no obvious sequence motif shared by all peptides. Four of the peptides appeared to be immunodominant IgE-binding epitopes in that they were recognized by serum from more than 80% of the patients tested and bound more IgE than any of the other Ara h 1 epitopes. Mutational analysis of the immunodominant epitopes revealed that single amino acid changes within these peptides had dramatic effects on IgE-binding characteristics. The identification and determination of the IgE-binding capabilities of core amino acids in epitopes on the Ara h 1 protein will make it possible to address the pathophysiologic and immunologic mechanisms regarding peanut hypersensitivity reactions specifically and food hypersensitivity in general.

L15 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2002 ACS
1996:565773 Document No. 125:219989 Reinvestigation of the major **peanut allergen** Ara h 1 on the molecular level. Buschmann, Liselotte; Petersen, Arnd; Schlaak, Max; Becker, Wolf-Meinhard (Division Allergology, Research Institute Borstel, Germany). Monogr. Allergy, 32(Highlights in Food Allergy), 92-98 (English) 1996. CODEN: MOALAR. ISSN: 0077-0760.

AB Studies on **peanut allergens** resulted in some inconsistent findings. Thus, the authors established monoclonal antibodies directed against peanut ext. aimed at the 66-kD allergen which was identified as a major allergen, Ara h 1. The monoclonal antibody PN-t was found to be species-specific with a reactivity to this protein. In this study, the antigenic and allergenic structures of **peanut allergens** were reinvestigated by 2D-PAGE with the aid of patients' sera, monoclonal antibodies, and lectins. The main reactivity of IgE was found in the 66-kD region. The Ara h 1 allergen was identified as Con A (Con A)-reactive glycoprotein. Amino acid compn. and sequence studies showed that Ara h 1 belongs to vicilins. Ara h 1 consists of at least 16 isoallergens , and at least 2 of the isoforms slightly differ in mol. wt. Moreover, Ara h 1 forms dimers and trimers of the isoallergens. It was concluded that Ara h 1 and the Con A-reactive allergen described by Barnett D. and Howden MEH (1986) are identical.

=> s Ara h2

L16 28 ARA H2

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L17 12 DUP REMOVE L16 (16 DUPLICATES REMOVED)

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L17 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2002 ACS
2001:380762 Document No. 135:1229 Isolation and sequence of a full length genomic clone for allergen **Ara h2** and down-regulation and silencing of allergen genes in transgenic peanut seeds. Dodo, Hortense W.; Arntzen, Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University, USA). PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31657 20001120. PRIORITY: US 1999-PV167255 19991119.

AB An allergen-free transgenic peanut seed is produced by recombinant methods. Peanut plants are transformed with multiple copies of each of the allergen genes, or fragments thereof, to suppress gene expression and allergen protein prodn. Alternatively, peanut plants are transformed with peanut allergen antisense genes introduced into the peanut genome as antisense fragments, sense fragments, or combinations of both antisense and sense fragments. Peanut transgenes are under the control of the 35S promoter, or the promoter of the **Ara h2** gene to produce antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing allergen protein prodn. in peanut plants. A full length genomic clone for allergen **Ara h2** is isolated and sequenced. The ORF is 622 nucleotides long. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One polyadenilation signal is identified at position 951. Six addnl. stop codons are obsd. A promoter region was revealed contg. a putative TATA box located at position -72. Homologous regions were identified between **Ara h2**, h6, and h7, and between Ara h3 and h4, and between Ara h1P41B and Ara h1P17. The homologous regions will be used for the screening of peanut genomic library to isolate all peanut allergen genes and for down-regulation and silencing of multiple peanut allergen genes.

L17 ANSWER 2 OF 12 MEDLINE DUPLICATE 1
2001248467 Document Number: 21192707. PubMed ID: 11274350. A strategy for the identification of proteins targeted by thioredoxin. Yano H; Wong J H; Lee Y M; Cho M J; Buchanan B B. (Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Apr 10) 98 (8) 4794-9. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Thioredoxins are 12-kDa proteins functional in the regulation of cellular processes throughout the animal, plant, and microbial kingdoms. Growing evidence with seeds suggests that an h-type of thioredoxin, reduced by NADPH via NADP-thioredoxin reductase, reduces disulfide bonds of target proteins and thereby acts as a wakeup call in germination. A better understanding of the role of thioredoxin in seeds as well as other systems could be achieved if more were known about the target proteins. To this end, we have devised a strategy for the comprehensive identification of proteins targeted by thioredoxin. Tissue extracts incubated with reduced thioredoxin are treated with a fluorescent probe (monobromobimane) to

label sulfhydryl groups. The newly labeled proteins are isolated by conventional two-dimensional electrophoresis: (i) nonreducing/reducing or (ii) isoelectric focusing/reducing SDS/PAGE. The isolated proteins are identified by amino acid sequencing. Each electrophoresis system offers an advantage: the first method reveals the specificity of thioredoxin in the reduction of intramolecular vs. intermolecular disulfide bonds, whereas the second method improves the separation of the labeled proteins. By application of both methods to peanut seed extracts, we isolated at least 20 thioredoxin targets and identified 5-three allergens (**Ara h2**, Ara h3, and Ara h6) and two proteins not known to occur in peanut (desiccation-related and seed maturation protein). These findings open the door to the identification of proteins targeted by thioredoxin in a wide range of systems, thereby enhancing our understanding of its function and extending its technological and medical applications.

L17 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2002 ACS
2001:295491 Document No. 135:151859 Detection of peanut allergens in breast milk of lactating women. Vadas, Peter; Wai, Yvonne; Burks, Wesley; Perelman, Boris (Division of Allergy and Clinical Immunology, St Michael's Hospital, University of Toronto, Toronto, ON, Can.). JAMA, J. Am. Med. Assoc., 285(13), 1746-1748 (English) 2001. CODEN: JAMAAP. ISSN: 0098-7484. Publisher: American Medical Association.

AB Most individuals who react to peanuts do so on their 1st known exposure. A potential but unproven route of occult exposure resulting in sensitization to peanut is via breast milk during lactation. To investigate the ability of maternal dietary peanut protein to pass into breast milk during lactation. Clin. investigation conducted at 2 North American hospitals from Mar. 1999 to Oct. 2000 including 23 healthy, lactating women aged 21-35 yr. Each woman consumed 50 g of dry roasted peanuts, after which breast milk samples were collected at hourly intervals. Presence in breast milk of total peanut protein, analyzed by a sandwich ELISA, and 2 major peanut allergens, Ara h 1 and Ara h 2, detected by immunoblot anal. Peanut protein was detected in 11 of 23 subjects. It was detected in 10 subjects within 2 h of ingestion and in 1 subject within 6 h. The median peak peanut protein concn. in breast milk was 200 ng/mL (mean, 222 ng/mL; range, 120-430 ng/mL). Both major peanut allergens Ara h 1 and Ara h 2 were detected. Conclusions Peanut protein is secreted into breast milk of lactating women following maternal dietary ingestion. Exposure to peanut protein during breastfeeding is a route of occult exposure that may result in sensitization of at-risk infants.

L17 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2002 ACS
2001:493625 Document No. 135:287805 Effects of cooking methods on peanut allergenicity. Beyer, Kirsten; Morrow, Ellen; Li, Xiu-Min; Bardina, Ludmilla; Bannon, Gary A.; Burks, A. Wesley; Sampson, Hugh A. (Division of Pediatric Allergy & Immunology and Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine, New York, NY, 10029-6574, USA). J. Allergy Clin. Immunol., 107(6), 1077-1081 (English) 2001. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..

AB Allergy to peanut is a significant health problem. Interestingly, the prevalence of peanut allergy in China is much lower than that in the United States, despite a high rate of peanut consumption in China. In China, peanuts are commonly fried or boiled, whereas in the United States peanuts are typically dry roasted. The aim of this study was to examine whether the method of prepg. peanuts could be a factor in the disparity of allergy prevalence between the 2 countries. Two varieties of peanuts grown in the United States were roasted, boiled, or fried. Proteins were analyzed by using SDS-PAGE and immunoblotting. Allergenicity was compared by using immunolabeling with sera from 8 patients with peanut allergy. The protein fractions of both varieties of peanuts were altered to a similar degree by frying or boiling. Compared with roasted peanuts, the relative amt. of Ara h 1 was reduced in the fried and boiled prepns., resulting in a significant redn. of IgE-binding intensity. In addn., there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried

and boiled peanuts compared with that in roasted peanuts, even though the protein amts. were similar in all 3 prepns. The methods of frying or boiling peanuts, as practiced in China, appear to reduce the allergenicity of peanuts compared with the method of dry roasting practiced widely in the United States. Roasting uses higher temps. that apparently increase the allergenic property of peanut proteins and may help explain the difference in prevalence of peanut allergy obsd. in the 2 countries.

L17 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2002 ACS
2001:363205 Document No. 136:101378 Isolation and molecular characterization of the first genomic clone of a major peanut allergen, Ara h 2. Viquez, Olga M.; Summer, Cathrine G.; Dodo, Hortense W. (Department of Food and Animal Sciences, Alabama A and M University, Normal, AL, 35762, USA). Journal of Allergy and Clinical Immunology, 107(4), 713-717 (English) 2001. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..
AB Peanuts have been identified as potent food allergens responsible for life-threatening IgE reactions among hypersensitive individuals. With the current increase of peanut allergies, there is an urgent need to molecularly characterize the genes encoding the target proteins and to understand the nature of their regulation. The objectives of this study were to isolate, sequence, and characterize at least one full-length genomic clone encoding the major peanut allergen Ara h 2. A peanut genomic library, constructed in a Lambda Fix II vector, was screened with an 80-bp oligonucleotide probe constructed on the basis of the 5' end of a published Ara h 2 cDNA partial sequence. One putative pos. lambda clone was isolated, digested with BamHI to release its 16-kb insert, and confirmed by dot blot and Southern hybridization. The pos. clone was subcloned in pBluescript SK+ vector, sequenced, and characterized. Sequence anal. revealed a full-length genomic clone with an open reading frame starting with an initiation codon (ATG) at position 1 and ending with a termination codon (TGA) at position 622. One putative polyadenylation signal (AATAAA) is identified at positions 951 in the 3' untranslated region, and 6 addnl. stop codons are located at positions 628, 769, 901, 946, 967, and 982 downstream from the start codon. In the 5' promoter region, a putative TATA box (TATTATTA) is located at position -72 upstream from the start codon. The deduced amino acid sequence has 207 residues and includes a putative signal peptide of 21 residues. The results reveal for the first time information on the structure of a major peanut allergen, Ara h 2. Comparison of the cDNA and genomic sequences revealed the absence of an intron but the presence of 2 isoforms of Ara h 2 or different members of the same gene family.

L17 ANSWER 6 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
2001:187787 Document No.: PREV200100187787. Investigation of the use of ISS-linked **Ara h2** for the treatment of peanut-induced allergy. Srivastava, K. (1); Li, X.-M.; Bannon, G. A.; Burks, A. W.; Eiden, J.; Vannest, G.; Tuck, R.; Rodriguez, R.; Sampson, H. A.. (1) Mount Sinai School of Medicine, New York, NY USA. Journal of Allergy and Clinical Immunology, (February, 2001) Vol. 107, No. 2, pp. S233. print. Meeting Info.: 57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology New Orleans, Louisiana, USA March 16-21, 2001 ISSN: 0091-6749. Language: English. Summary Language: English.

L17 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:178088 Document No.: PREV200100178088. Role of conformational and linearized epitopes in the achievement of tolerance in peanut allergy. Ellman, Lisa Kim (1); Beyer, Kirsten (1); Bardina, Ludmilla (1); Jarvinen, Kirsi-Marjut (1); Bannon, Gary A.; Burks, Wesley; Sampson, Hugh A. (1). (1) Mount Sinai School of Medicine, New York, NY USA. Journal of Allergy and Clinical Immunology, (February, 2001) Vol. 107, No. 2, pp. S139. print. Meeting Info.: 57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology New Orleans, Louisiana, USA March 16-21, 2001 ISSN: 0091-6749. Language: English. Summary Language: English.

L17 ANSWER 8 OF 12 MEDLINE DUPLICATE 3
2001322186 Document Number: 21097438. PubMed ID: 11167950. Polyisotypic antipeanut-specific humoral responses in peanut-allergic individuals. Kolopp-Sarda M N; Moneret-Vautrin D A; Gobert B; Kanny G; Guerin L; Faure G C; Bene M C. (Laboratoire d'Immunologie, Faculte de Medecine & CHU de Nancy, 54500 Vandoeuvre-les-Nancy, France.) CLINICAL AND EXPERIMENTAL ALLERGY, (2001 Jan) 31 (1) 47-53. Journal code: CEB; 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Peanut-containing food products may induce severe clinical reactions in sensitized subjects, and high levels of antipeanut IgE have been reported in the literature. Immunotherapy, proposed for the prevention of severe accidents, is often ill-tolerated and only partly efficient. This could be due to the spontaneous development of polyisotypic antipeanut antibodies. OBJECTIVE: To appreciate the presence and reactivity of other isotypes other than IgE of peanut-specific antibodies in serum samples from peanut-sensitized subjects. METHODS: Serum samples were obtained from 20 non-sensitized subjects and 23 sensitized patients divided in three groups according to their response to peanut oral challenge (no response or response to high or low doses, respectively). Peanut-specific IgG, IgG subclasses, IgA and IgM were assayed using an ELISA, and their reactivity against peanut proteins tested using Western Blot. RESULTS: A large dispersion of antipeanut antibody levels was observed in the three groups of patients, high levels of IgG, IgG1, IgG4 and IgA usually correlating with highly positive radioallergosorbent test (RAST). Such high levels were observed at onset in four patients who underwent peanut immunotherapy who had side effects and poor efficiency. Western blotting demonstrated that the polyisotypic response observed was directed to several peanut antigens, including the major allergens, Ara h1 and **Ara h2**. CONCLUSION: Peanut-sensitized patients who spontaneously develop specific IgE, display polyisotypic-specific antibody responses, whatever their response to oral challenge. This might explain the poor efficiency of peanut rush immunotherapy attempts.

L17 ANSWER 9 OF 12 MEDLINE DUPLICATE 4
1999172246 Document Number: 99172246. PubMed ID: 10072557. Strain-dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. Li X; Huang C K; Schofield B H; Burks A W; Bannon G A; Kim K H; Huang S K; Sampson H A. (Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029, USA.. Xiu-min_li@smtplink.mssm.edu) . JOURNAL OF IMMUNOLOGY, (1999 Mar 1) 162 (5) 3045-52. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB To investigate the potential application of allergen gene immunization in the modulation of food allergy, C3H/HeSn (C3H) mice received i.m. injections of pAra h2 plasmid DNA encoding one of the major peanut allergens, **Ara h2**. Three weeks following pDNA immunization, serum **Ara h2**-specific IgG2a, IgG1, but not IgE, were increased significantly in a dose-dependent manner. IgG1 was 30-fold higher in multiply compared with singly immunized mice. **Ara h2** or peanut protein injection of immunized mice induced anaphylactic reactions, which were more severe in multiply immunized mice. Heat-inactivated immune serum induced passive cutaneous anaphylaxis, suggesting that anaphylaxis in C3H mice was mediated by IgG1. IgG1 responses were also induced by intradermal injection of pAra h2, and by i.m. injection of pOMC, the plasmid DNA encoding the major egg allergen protein, ovomucoid. To elucidate whether the pDNA immunization-induced anaphylaxis was a strain-dependent phenomenon, AKR/J and BALB/c mice also received multiple i.m. pAra h2 immunizations. Injection of peanut protein into these strains at weeks 3 or 5 following immunization did not induce reactions. Although IgG2a was increased significantly from week 2 in AKR/J mice and from week 4 in BALB/c mice and remained elevated for at least 6 wk, no IgG1 or IgE was detected. These results indicate that the type of

immune responses to pDNA immunization in mice is strain dependent. Consequently, models for studying human allergen gene immunization require careful selection of suitable strains. In addition, this suggests that similar interindividual variation is likely in humans.

L17 ANSWER 10 OF 12 MEDLINE DUPLICATE 5
1998339794 Document Number: 98339794. PubMed ID: 9677140. Identification and partial characterization of multiple major allergens in peanut proteins. de Jong E C; Van Zijverden M; Spanhaak S; Koppelman S J; Pellegrom H; Penninks A H. (TNO Nutrition and Food Research Institute, Immunotoxicology group, Zeist, The Netherlands.) CLINICAL AND EXPERIMENTAL ALLERGY, (1998 Jun) 28 (6) 743-51. Journal code: CEB; 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Peanuts are a major cause of food allergies both in children as in adults which can induce an anaphylactic shock. The identification and characterization of peanut allergens could lead to more insight into the mechanism and contribute to the improvement of diagnostic tests and treatment for peanut allergy. OBJECTIVE: In the present study, the peanut protein-specific immunoglobulin concentrations as well as their recognition of the various peanut proteins or protein subunits was determined in the plasma of peanut-allergic (PA) and non-allergic (NA) individuals. Moreover, two peanut allergens were characterized in more detail to confirm them as the earlier described Ara h1 and **Ara h2**. METHODS: The presence of Ig-binding sites in peanut proteins was studied by immunoblotting assays whereas the concentrations of peanut-specific Ig was determined by ELISA. RESULTS: Peanut proteins were found to contain multiple binding sites for immunoglobulins. Of these proteins, six were recognized by peanut-specific IgE present in more than 50% of the plasma samples of the PA group. Their molecular weights were approximately 44, 40, 33, 21, 20 and 18 kDa. The last three protein bands were recognized by peanut-specific IgE present in more than 70% of the PA plasma samples and were thought to contain **Ara h2**.
This allergen as well as another protein that was thought to be Ara h1, which was not recognized by the majority of the patients' IgE-containing plasma samples, were isolated and the N terminal amino acid sequence was determined. Peanut protein-specific IgA, IgM, IgG and IgG-subclasses showed a more diverse recognition pattern of peanut protein in the PA group compared to the NA group. No differences were found in the plasma concentrations of peanut protein-specific immunoglobulins of the various classes between the PA and NA group. CONCLUSIONS: From the present study, we conclude that peanuts contain multiple allergens, of which six can be described as major allergens, **Ara h2** included. In our population Ara h1 is not a major allergen. The recognition of peanut proteins by immunoglobulins is more diverse in PA individuals compared with NA individuals which, however, is not substantiated in the concentrations of peanut-specific immunoglobulins in plasma, other than IgE.

L17 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2002 ACS
1996:612305 Document No. 125:273253 Stability of food allergens to digestion in vitro. Astwood, James D.; Leach, John N.; Fuchs, Roy L. (Monsanto Co., St Louis, MO, 63198, USA). Nat. Biotechnol., 14(10), 1269-1273 (English) 1996. CODEN: NABIF9. ISSN: 1087-0156.

AB An integral part of the safety assessment of genetically modified plants is consideration of possible human health effects, esp. food allergy. Prospective testing for allergenicity of proteins obtained from sources with no prior history of causing allergy has been difficult because of the absence of valid methods and models. Food allergens may share physicochem. properties that distinguish them from nonallergens, properties that may be used as a tool to predict the inherent allergenicity of proteins newly introduced into the food supply by genetic engineering. One candidate property is stability to digestion. We have systematically evaluated the stability of food allergens that are active

via the gastrointestinal tract in a simple model of gastric digestion, emphasizing the major allergens of plant-derived foods such as legumes (peanuts and soybean). Important food allergens were stable to digestion in the gastric model (simulated gastric fluid). For example, soybean .beta.-conglycinin was stable for 60 min. In contrast, nonallergenic food proteins, such as spinach ribulose bis-phosphate carboxylase/oxygenase, were digested in simulated gastric fluid within 15 s. The data are consistent with the hypothesis that food allergens must exhibit sufficient gastric stability to reach the intestinal mucosa where absorption and sensitization (development of atopy) can occur. Thus, the stability to digestion is a significant and valid parameter that distinguishes food allergens from nonallergens.

L17 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6

1986:454896 Document No.: BA82:111738. HYDROGEN EVOLUTION AND RELATIVE EFFICIENCY IN CHICK PEA CICER-ARIETINUM EFFECT OF RHIZOBIUM STRAIN HOST CULTIVAR AND TEMPERATURE. SINDHU S S; DADARWAL K R; DAHIYA B S. DEP. OF MICROBIOL., HARYANA AGRICULTURAL UNIV., HISSAR 125 004, INDIA.. INDIAN J EXP BIOL, (1986) 24 (7), 416-420. CODEN: IJEBA6. ISSN: 0019-5189. Language: English.

AB Effect of Rhizobium strains, host cultivars, plant age and temperature on acetylene reduction activity (**ARA**), **H2** evolution and relative efficiency (RE) were studied in C. arietinum. The Rhizobium strains nodulating this legume were observed to be of Hup- phenotype. At soil temperature prevailing during growth and active nitrogen fixation stage an average RE of 0.81 was observed in cultivar C235 up to day 90 of plant growth by inoculating different Hup- strains. Variance due to Rhizobium strains in RE was not significant. Relative ARA values were initially low at day 45 of plant growth which increased at day 60 followed by a slight decline at day 90. However, no significant differences in RE values were observed due to plant age except in case of those strains where early degeneration of nodules started, resulting into decrease in RE. Also, Rhizobium strains differing in effectivity, showing difference in plant dry weight ratios from less than 3.0 to more than 8.0, did not differ significantly in RE. Host cultivars did not change significantly RE of different Rhizobium strains. Effects of temperature on ARA and H2 evolution were studied in eight cultivars grown under field conditions and nodulated with native Rhizobium strains. The average RE when measured at 20.degree. C, in these cultivars was above 0.8 from day 60 to 105 of plant growth. Hydrogen uptake activity was absent in nodules of all cultivars. At 25.degree. C, RE decreased in all cultivars, mainly due to decrease in relative ARA. Cultivar effect on RE was more pronounced at 25.degree. C as two of the cultivars H82-2 and H75-35 showed significantly low RE at this temperature. H2 evolution in C2H2 atmosphere was increased 2-fold at 25.degree. C as compared to 20.degree. C in nodules of all the cultivars.

=> s ara h3
L18 12 ARA H3

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L19 7 DUP REMOVE L18 (5 DUPLICATES REMOVED)

=> d l19 1-7 cbib abs

L19 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2002 ACS

2001:380762 Document No. 135:1229 Isolation and sequence of a full length genomic clone for allergen Ara h2 and down-regulation and silencing of allergen genes in transgenic peanut seeds. Dodo, Hortense W.; Arntzen, Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University, USA). PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,

CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31657 20001120. PRIORITY: US 1999-PV167255 19991119.

AB An allergen-free transgenic peanut seed is produced by recombinant methods. Peanut plants are transformed with multiple copies of each of the allergen genes, or fragments thereof, to suppress gene expression and allergen protein prodn. Alternatively, peanut plants are transformed with peanut allergen antisense genes introduced into the peanut genome as antisense fragments, sense fragments, or combinations of both antisense and sense fragments. Peanut transgenes are under the control of the 35S promoter, or the promoter of the Ara h2 gene to produce antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing allergen protein prodn. in peanut plants. A full length genomic clone for allergen Ara h2 is isolated and sequenced. The ORF is 622 nucleotides long. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One polyadenilation signal is identified at position 951. Six addnl. stop codons are obsd. A promoter region was revealed contg. a putative TATA box located at position -72. Homologous regions were identified between Ara h2, h6, and h7, and between **Ara h3** and h4, and between Ara h1P41B and Ara h1P17. The homologous regions will be used for the screening of peanut genomic library to isolate all peanut allergen genes and for down-regulation and silencing of multiple peanut allergen genes.

L19 ANSWER 2 OF 7 MEDLINE DUPLICATE 1

2001248467 Document Number: 21192707. PubMed ID: 11274350. A strategy for the identification of proteins targeted by thioredoxin. Yano H; Wong J H; Lee Y M; Cho M J; Buchanan B B. (Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Apr 10) 98 (8) 4794-9. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Thioredoxins are 12-kDa proteins functional in the regulation of cellular processes throughout the animal, plant, and microbial kingdoms. Growing evidence with seeds suggests that an h-type of thioredoxin, reduced by NADPH via NADP-thioredoxin reductase, reduces disulfide bonds of target proteins and thereby acts as a wakeup call in germination. A better understanding of the role of thioredoxin in seeds as well as other systems could be achieved if more were known about the target proteins. To this end, we have devised a strategy for the comprehensive identification of proteins targeted by thioredoxin. Tissue extracts incubated with reduced thioredoxin are treated with a fluorescent probe (monobromobimane) to label sulfhydryl groups. The newly labeled proteins are isolated by conventional two-dimensional electrophoresis: (i) nonreducing/reducing or (ii) isoelectric focusing/reducing SDS/PAGE. The isolated proteins are identified by amino acid sequencing. Each electrophoresis system offers an advantage: the first method reveals the specificity of thioredoxin in the reduction of intramolecular vs. intermolecular disulfide bonds, whereas the second method improves the separation of the labeled proteins. By application of both methods to peanut seed extracts, we isolated at least 20 thioredoxin targets and identified 5-three allergens (Ara h2, **Ara h3**, and Ara h6) and two proteins not known to occur in peanut (desiccation-related and seed maturation protein). These findings open the door to the identification of proteins targeted by thioredoxin in a wide range of systems, thereby enhancing our understanding of its function and extending its technological and medical applications.

L19 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2002 ACS

2001:493625 Document No. 135:287805 Effects of cooking methods on peanut allergenicity. Beyer, Kirsten; Morrow, Ellen; Li, Xiu-Min; Bardina, Ludmilla; Bannon, Gary A.; Burks, A. Wesley; Sampson, Hugh A. (Division of Pediatric Allergy & Immunology and Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine, New York, NY, 10029-6574, USA). J. Allergy Clin. Immunol., 107(6), 1077-1081 (English) 2001. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..

AB Allergy to peanut is a significant health problem. Interestingly, the prevalence of peanut allergy in China is much lower than that in the United States, despite a high rate of peanut consumption in China. In China, peanuts are commonly fried or boiled, whereas in the United States peanuts are typically dry roasted. The aim of this study was to examine whether the method of prepg. peanuts could be a factor in the disparity of allergy prevalence between the 2 countries. Two varieties of peanuts grown in the United States were roasted, boiled, or fried. Proteins were analyzed by using SDS-PAGE and immunoblotting. Allergenicity was compared by using immunolabeling with sera from 8 patients with peanut allergy. The protein fractions of both varieties of peanuts were altered to a similar degree by frying or boiling. Compared with roasted peanuts, the relative amt. of Ara h 1 was reduced in the fried and boiled prepns., resulting in a significant redn. of IgE-binding intensity. In addn., there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts compared with that in roasted peanuts, even though the protein amts. were similar in all 3 prepns. The methods of frying or boiling peanuts, as practiced in China, appear to reduce the allergenicity of peanuts compared with the method of dry roasting practiced widely in the United States. Roasting uses higher temps. that apparently increase the allergenic property of peanut proteins and may help explain the difference in prevalence of peanut allergy obsd. in the 2 countries.

L19 ANSWER 4 OF 7 MEDLINE

2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell

proliferation. CONCLUSIONS: These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy. Copyright 2001 S. Karger AG, Basel

L19 ANSWER 5 OF 7 MEDLINE DUPLICATE 2

2001119281 Document Number: 21066595. PubMed ID: 11146387. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. Beardslee T A; Zeece M G; Sarath G; Markwell J P. (Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA.. beardsl@yahoo.com) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Dec) 123 (4) 299-307. Journal code: BJ7. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: The identification of IgE epitopes for proteins is the first step in understanding the interaction of allergens with the immune system. Proteins from the legume family have shown in vitro cross-reactivity in IgE-binding assays, but this cross-reactivity is rarely clinically significant. Resolution of this discrepancy requires IgE epitope mapping of legume family protein allergens. METHODS: We constructed six fusion proteins representing overlapping regions of soybean glycinin G1 acidic chain. These fusion proteins were used in immunoblotting and a novel sandwich ELISA with pooled sera from soy-allergic individuals to reveal a common IgE-binding region. This region was the focus for IgE epitope mapping using overlapping synthetic peptides. RESULTS: Data from the fusion protein experiments revealed an IgE-binding region consisting of residues F192-I265. Analysis of the overlapping synthetic peptides to this region indicated that IgE epitopes to glycinin G1 acidic chain consist of residues G217-V235 and G253-I265. The epitopes identified for glycinin G1 acidic chain are homologous to IgE epitopes previously identified for the peanut allergen Ara h 3 [1]. However, residues identified by alanine scanning in the peanut epitopes as being important for IgE binding are different in the natural soybean epitopes. CONCLUSIONS: The IgE epitopes identified for glycinin G1 acidic chain apparently represent an allergenic region of several legume family seed storage proteins. Our findings indicate that the identification of IgE epitopes and structural analysis of legume family proteins will provide valuable information to the study of food allergies. Copyright 2000 S. Karger AG, Basel

L19 ANSWER 6 OF 7 MEDLINE

1999146968 Document Number: 99146968. PubMed ID: 10021462. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. Rabjohn P; Helm E M; Stanley J S; West C M; Sampson H A; Burks A W; Bannon G A. (Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1999 Feb) 103 (4) 535-42. Journal code: HS7; 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Peanut allergy is a significant IgE-mediated health problem because of the increased prevalence, potential severity, and chronicity of the reaction. Following our characterization of the two peanut allergens Ara h 1 and Ara h 2, we have isolated a cDNA clone encoding a third peanut allergen, Ara h 3. The deduced amino acid sequence of Ara h 3 shows homology to 11S seed-storage proteins. The recombinant form of this protein was expressed in a bacterial system and was recognized by serum IgE from approximately 45% of our peanut-allergic patient population. Serum IgE from these patients and overlapping, synthetic peptides were used to map the linear, IgE-binding epitopes of Ara h 3. Four epitopes, between 10 and 15 amino acids in length, were found within the primary sequence, with no obvious sequence motif shared by the peptides. One epitope is recognized by all Ara h 3-allergic patients. Mutational analysis of the epitopes revealed that single amino acid changes within these peptides could lead to a reduction or loss of IgE binding. By determining which amino acids are critical for IgE binding, it might be possible to alter the Ara h 3 cDNA to encode a protein with a reduced IgE-binding capacity. These results will enable the design of improved diagnostic and therapeutic approaches

for food-hypersensitivity reactions.

L19 ANSWER 7 OF 7 MEDLINE
1999406463 Document Number: 99406463. PubMed ID: 10474031. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. Kleber-Janke T; Crameri R; Appenzeller U; Schlaak M; Becker W M. (Research Center Borstel, Germany.. tkleber@fz-borstel.de) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1999 Aug) 119 (4) 265-74. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Peanut kernels contain many allergens able to elicit IgE-mediated type 1 allergic reactions in sensitized individuals. Sera from sensitized patients recognize variable patterns of IgE-binding proteins. The identification of the IgE-binding proteins of peanut extract would faciliate improvement of diagnostic and immunotherapeutic approaches as well as development of sensitive test systems for the detection of hidden peanut allergens present as additives in various industrial food products and the investigation of their stability during processing of food products. METHODS: We applied the pJuFo cloning system based on the phage surface display of functional cDNA expression products to clone cDNAs encoding peanut allergens. Sera (n = 40) of peanut-allergic individuals were selected according to case history, radioallergosorbent test and immunoblot analysis to demonstrate IgE binding towards the newly identified recombinant allergens. RESULTS: In addition to the known allergens Ara h 1 and Ara h 2 we were able to identify four allergens with estimated molecular weights of 36, 16, 14.5 and 14 kDa. Three of them formally termed Ara h 4, Ara h6 and Ara h 7 show significant sequence similarities to the family of seed storage proteins and the fourth (Ara h 5) corresponds to the well-known plant allergen profilin. Immunoblotting of the six expressed recombinant allergens with 40 patients sera shows 14 individual recognition patterns and the following frequency of specific IgE binding: Ara h 1 was recognized by 65%, Ara h 2 by 85%, Ara h 4 by 53%, Ara h 5 by 13%, Ara h 6 by 38% and Ara h 7 by 43% of the selected sera. CONCLUSIONS: All of the selected peanut-positive sera can detect at least one of the six identified recombinant allergens which can be used to establish individual patients' reactivity profiles. A comparison of these profiles with the clinical data will possibly allow a further insight into the relationship between clinical severity of the symptoms and specific IgE levels towards the six peanut allergens.

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L20       1901 BANNON G?/AU OR BURKS W?/AU OR SAMPSON H?/AU OR SOSIN H?/AU

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L21        130 L20 AND PEANUT ALLERGEN

=> dup remove l21
PROCESSING COMPLETED FOR L21
L22         80 DUP REMOVE L21 (50 DUPLICATES REMOVED)

=> s l22 and polynucleotide
L23          0 L22 AND POLYNUCLEOTIDE

=> s l22 and cDNA
L24         11 L22 AND CDNA

=> dup remove l24
PROCESSING COMPLETED FOR L24
L25         11 DUP REMOVE L24 (0 DUPLICATES REMOVED)

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L25 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2002 ACS
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2001:416973 Document No. 135:45198 Prevention of an anaphylactic response to food allergens. **Bannon, Gary A.**; **Burks, Wesley A.**; Caplan, Michael J.; **Sampson, Hugh**; **Sosin, Howard** (Panacea Pharmaceuticals, LLC, USA; University of Arkansas; Mount Sinai School of Medicine of New York). PCT Int. Appl. WO 2001040264 A2 20010607, 100 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33124 20001206. PRIORITY: US 1999-455294 19991206; US 2000-PV213765 20000623; US 2000-PV235797 20000927.

AB The authors disclose methods for reducing allergic responses in individuals sensitive to one or more food antigens. In general, desensitization is achieved by administration of fragments of antigens characterized by a reduced ability to bind to their cognate IgE. In one example, mice were sensitized to **peanut allergens** by intragastric feeding. Administration of peptide fragments of Ara h 2, or an allergen mutein with altered IgE binding sites, abrogated an increase in IgE levels and anaphylactic sequelae.

L25 ANSWER 2 OF 11 MEDLINE

2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major **peanut allergens** for use in immunotherapy. **Bannon G A**; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; **Sampson H A**; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The **cDNA** clones for three major **peanut allergens**, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen **cDNA** clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified **peanut allergens** were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. CONCLUSIONS: These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy. Copyright 2001 S. Karger AG, Basel

L25 ANSWER 3 OF 11 MEDLINE

1999146968 Document Number: 99146968. PubMed ID: 10021462. Molecular cloning and epitope analysis of the **peanut allergen** Ara h 3. Rabjohn P; Helm E M; Stanley J S; West C M; **Sampson H A** ; Burks A W; **Bannon G A**. (Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1999 Feb) 103 (4) 535-42. Journal code: HS7; 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Peanut allergy is a significant IgE-mediated health problem because of the increased prevalence, potential severity, and chronicity of the reaction. Following our characterization of the two **peanut allergens** Ara h 1 and Ara h 2, we have isolated a **cDNA** clone encoding a third **peanut allergen**, Ara h 3. The deduced amino acid sequence of Ara h 3 shows homology to 11S seed-storage proteins. The recombinant form of this protein was expressed in a bacterial system and was recognized by serum IgE from approximately 45% of our peanut-allergic patient population. Serum IgE from these patients and overlapping, synthetic peptides were used to map the linear, IgE-binding epitopes of Ara h 3. Four epitopes, between 10 and 15 amino acids in length, were found within the primary sequence, with no obvious sequence motif shared by the peptides. One epitope is recognized by all Ara h 3-allergic patients. Mutational analysis of the epitopes revealed that single amino acid changes within these peptides could lead to a reduction or loss of IgE binding. By determining which amino acids are critical for IgE binding, it might be possible to alter the Ara h 3 **cDNA** to encode a protein with a reduced IgE-binding capacity. These results will enable the design of improved diagnostic and therapeutic approaches for food-hypersensitivity reactions.

L25 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:154703 Document No.: PREV199800154703. Glycinin, a third major **peanut allergen** identified by soy-adsorbed serum IgE from peanut sensitive individuals. Rabjohn, P.; West, C. M.; Helm, E.; Helm, R.; Stanley, J. S.; Huang, S. K.; **Sampson, H.**; Burks, A. W.; **Bannon, G. A.**. Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S240. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Washington, DC, USA March 13-18, 1998 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L25 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS
1998:141656 Characterization and epitope analysis of ARA h 3, a glycinin involved in peanut hypersensitivity.. Helm, Erica M.; Rabjohn, Pat A.; Stanley, J. Steven; West, C. Michael; Huang, S. K.; **Sampson, H.**; Burks, A. Wesley; **Bannon, Gary A.** (Department Chemistry, Hendrix College, Conway, AR, 72032, USA). Book of Abstracts, 215th ACS National Meeting, Dallas, March 29-April 2, CHED-179. American Chemical Society: Washington, D. C. (English) 1998. CODEN: 65QTAA.

AB Peanut allergy is a major health concern due to the severity of the allergic reaction, the lifelong persistence of the allergy, and the ubiquitous use of peanut as a protein supplement in processed foods. Using a previously unidentified **peanut allergen**, Ara h 3 **cDNA** clone was isolated, sequenced and found to be 1530 nucleotides and encoded a 510 amino acid protein. This sequence showed homol. to the glycinin family of seed storage proteins of common legumes. Synthetic peptides were used to det. which regions of the primary sequence served as linear B-cell epitopes for binding serum IgE from a population of peanut hypersensitivity patients. These epitopes were distributed evenly throughout the primary sequence and were six to ten amino acids in length. Further studies will be focused on identifying individual amino acids crit. for IgE binding. Once these amino acids are identified, it will be possible to mutate crit. residues to eliminate the ability of this protein to bind IgE.

L25 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS
1997:473729 Document No. 127:94502 Cloning, nucleotide and amino acid sequences, and immunoassays of **peanut allergens** causing hypersensitivity. Burks, A. Wesley, Jr.; Helm, Ricki M.; Cockrell, Gael; Stanley, J. Steven; **Bannon, Gary A.** (University of Arkansas, USA; Burks, A. Wesley, Jr.; Helm, Ricki M.; Cockrell, Gael; Stanley, J. Steven; Bannon, Gary A.). PCT Int. Appl. WO 9724139 A1 19970710, 352 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US15222 19960923. PRIORITY: US 1995-9455 19951229; US 1996-610424 19960304.

AB Crude Florunner exts. were fractioned by anion-exchange chromatog. using a step gradient. A protein peak which eluted at 10% NaCl and demonstrated intense IgE-binding was further analyzed by 2-dimensional SDS-PAGE/immunoblot anal. The majority of this fraction is a protein which has a mol. wt. of 17 kDa and a pI of 5.2. Sequencing data from the N-terminus revealed the following initial 9 amino acids: (*)-Q-Q-(*)-E-L-Q-D-L. Based on IgE-binding activity and no known amino acid sequence identity to other allergens, this allergen is designated Ara h II. Ara h II may be used to detect and quantify **peanut allergens** in foodstuffs. Serum IgE from patients with documented peanut hypersensitivity reactions and a peanut **cDNA** expression library were used to identify clones that encode **peanut allergens**. One of the major **peanut allergens**, Ara h I, was selected from these clones using Ara h I-specific oligonucleotides and PCR technol. The **cDNA** and deduced amino acid sequences are presented for Ara h I (a vicilin-like protein) and Ara h II (a conglutin-like protein). B-cell epitope mapping and monoclonal antibody prodn. allowed the development of efficient immunoassays, and the allergens can be used for vaccination therapy to treat peanut hypersensitivity in human patients.

L25 ANSWER 7 OF 11 MEDLINE
97330026 Document Number: 97330026. PubMed ID: 9186485. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major **peanut allergen** Ara h 2. Stanley J S; King N; Burks A W; Huang S K; **Sampson H**; Cockrell G; Helm R M; West C M; **Bannon G A**. (Department of Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1997 Jun 15) 342 (2) 244-53. Journal code: 6SK; 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB A major **peanut allergen**, Ara h 2, is recognized by serum IgE from > 90% of patients with peanut hypersensitivity. Biochemical characterization of this allergen indicates that it is a glycoprotein of approximately 17.5 kDa. Using N-terminal amino acid sequence data from purified Ara h 2, oligonucleotide primers were synthesized and used to identify a clone (741 bp) from a peanut **cDNA** library. This clone was capable of encoding a 17.5-kDa protein with homology to the conglutin family of seed storage proteins. The major linear immunoglobulin E (IgE)-binding epitopes of this allergen were mapped using overlapping peptides synthesized on an activated cellulose membrane and pooled serum IgE from 15 peanut-sensitive patients. Ten IgE-binding epitopes were identified, distributed throughout the length of the Ara h 2 protein. Sixty-three percent of the amino acids represented in the epitopes were either polar uncharged or apolar residues. In an effort to determine which, if any, of the 10 epitopes were recognized by the majority of patients with peanut hypersensitivity, each set of 10 peptides was probed

reaction technology. The Ara h I clone identified a 2.3-kb mRNA species on a Northern blot containing peanut poly (A)+ RNA. DNA sequence analysis of the cloned inserts revealed that the Ara h I allergen has significant homology with the vicilin seed storage protein family found in most higher plants. The isolation of the Ara h I clones allowed the synthesis of this protein in E. coli cells and subsequent recognition of this recombinant protein in immunoblot analysis using serum IgE from patients with peanut hypersensitivity. With the production of the recombinant peanut protein it will now be possible to address the pathophysiologic and immunologic mechanisms regarding peanut hypersensitivity reactions specifically and food hypersensitivity in general

L25 ANSWER 11 OF 11 MEDLINE
95337745 Document Number: 95337745. PubMed ID: 7613142. Isolation, identification, and characterization of clones encoding antigens responsible for peanut hypersensitivity. Burks A W; Cockrell G; Stanley J S; Helm R M; **Bannon G A**. (Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, USA.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1995 May-Jun) 107 (1-3) 248-50. Ref: 6. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB Peanut allergy is a significant health problem because of the frequency, the potential severity, and the chronicity of the allergic sensitivity. Serum IgE from patients with documented peanut hypersensitivity reactions and a peanut **cDNA** expression library were used to identify clones that encode **peanut allergens**. One of the major **peanut allergens**, Ara h I, was selected from these clones using Ara h I-specific oligonucleotides and polymerase chain reaction technology. The Ara h I clone identified a 2.3-kb mRNA species on a Northern blot containing peanut poly A+RNA. DNA sequence analysis of the cloned inserts revealed that the Ara h I allergen has significant homology with the vicilin seed storage protein family found in most higher plants. The isolation of the Ara h I clones allowed the synthesis of this protein in Escherichia coli cells and subsequent recognition of this recombinant protein in immunoblot analysis using serum IgE from patients with peanut hypersensitivity. With the production of the recombinant peanut protein it will now be possible to address the pathophysiologic and immunologic mechanisms regarding peanut hypersensitivity reactions specifically and food hypersensitivity in general.

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p?/au or shin d?/au or campadre c?/au
L26       7752 KING N?/AU OR MALEKI S?/AU OR CONNAUGHTON C?/AU OR KOPPER R?/AU
               OR RABJOHN P?/AU OR SHIN D?/AU OR CAMPADRE C?/AU

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L27         93 L26 AND PEANUT ALLERGEN

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PROCESSING COMPLETED FOR L27
L28          50 DUP REMOVE L27 (43 DUPLICATES REMOVED)

=> s l28 and cDNA
L29          6 L28 AND CDNA

=> dup remove l29
PROCESSING COMPLETED FOR L29
L30           6 DUP REMOVE L29 (0 DUPLICATES REMOVED)

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L30 ANSWER 1 OF 6 MEDLINE
2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering,

individually with serum IgE from 10 different patients. All of the patient sera tested recognized multiple epitopes. Three epitopes (aa27-36, aa57-66, and aa65-74) were recognized by all patients tested. In addition, these three peptides bound more IgE than all the other epitopes combined, indicating that they are the immunodominant epitopes of the Ara h 2 protein. Mutational analysis of the Ara h 2 epitopes indicate that single amino acid changes result in loss of IgE binding. Two epitopes in region aa57-74 contained the amino acid sequence DPYSP that appears to be necessary for IgE binding. These results may allow for the design of improved diagnostic and therapeutic approaches to peanut hypersensitivity.

L25 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS
1997:159532 Cloning of a portion of Ara h 3: A **peanut allergen**.. Helm, Erica M.; Rabjohn, P. A.; Burks, A. W.; **Sampson, H. A.**; **Bannon, G. A.** (Hendrix College, Conway, AR, 72032, USA). Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17, CHED-241. American Chemical Society: Washington, D. C. (English) 1997. CODEN: 64AOAA.
AB Four peanut proteins have been identified as major allergens in peanut hypersensitivity. This study involved the identification and sequencing of one of these proteins, Ara h 3. Sera from groups of allergic people were used to identify IgE binding proteins by Western blot anal. A .apprx. 14kD protein was isolated and a portion of it's amino acid sequence was used to derive oligonucleotide probes that were utilized as PCR primers and hybridization probes to clone the gene that encoded this protein. A **cDNA** clone carrying a 1200 bp insert was isolated. Northern blot anal. revealed that this insert hybridized to an .apprx.1.6 kb mRNA, indicating that the insert was not full length. DNA sequence anal. revealed that Ara h 3 was a glycinin, a seed storage protein. As a continuation of this study, the known sequence will be mutagenized to identify the amino acids that are important in causing peanut hypersensitivity.

L25 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2002 ACS
1997:474603 Document No. 127:175339 Peanut hypersensitivity: IgE binding characteristics of a recombinant Ara h I protein. Stanley, J. S.; Helm, R. M.; Cockrell, G.; Burks, A. W.; **Bannon, G. A.** (Departments of Pediatrics and Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA). Adv. Exp. Med. Biol., 409(New Horizons in Allergy Immunotherapy), 213-216 (English) 1996. CODEN: AEMBAP. ISSN: 0065-2598. Publisher: Plenum.
AB The Ara h I **peanut allergen** was cloned and partially characterized using serum IgE from peanut-hypersensitive individuals and IgE reactive clones isolated from a peanut **cDNA** expression library. Of 18 patients tested, 17 had IgE which recognized recombinant Ara h I. The major IgE binding domain(s) on the recombinant mol. were preliminarily mapped, indicating that there are multiple IgE epitopes on the Ara h I allergen.

L25 ANSWER 10 OF 11 MEDLINE
96013631 Document Number: 96013631. PubMed ID: 7560062. Recombinant **peanut allergen** Ara h I expression and IgE binding in patients with peanut hypersensitivity. Burks A W; Cockrell G; Stanley J S; Helm R M; **Bannon G A**. (Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock 72205, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1995 Oct) 96 (4) 1715-21. Journal code: HS7; 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB Peanut allergy is a significant health problem because of the frequency, the potential severity, and the chronicity of the allergic sensitivity. Serum IgE from patients with documented peanut hypersensitivity reactions and a peanut **cDNA** expression library were used to identify clones that encode **peanut allergens**. One of the major **peanut allergens**, Ara h I, was selected from these clones using Ara h I specific oligonucleotides and polymerase chain

characterization and in vitro efficacy of the major **peanut allergens** for use in immunotherapy. Bannon G A; Cockrell G; **Connaughton C**; West C M; Helm R; Stanley J S; **King N**; **Rabjohn P**; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The **cDNA** clones for three major **peanut allergens**, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen **cDNA** clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified **peanut allergens** were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. CONCLUSIONS: These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy. Copyright 2001 S. Karger AG, Basel

L30 ANSWER 2 OF 6 MEDLINE

1999146968 Document Number: 99146968. PubMed ID: 10021462. Molecular cloning and epitope analysis of the **peanut allergen** Ara h 3. **Rabjohn P**; Helm E M; Stanley J S; West C M; Sampson H A; Burks A W; Bannon G A. (Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1999 Feb) 103 (4) 535-42. Journal code: HS7; 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Peanut allergy is a significant IgE-mediated health problem because of the increased prevalence, potential severity, and chronicity of the reaction. Following our characterization of the two **peanut allergens** Ara h 1 and Ara h 2, we have isolated a **cDNA** clone encoding a third **peanut allergen**, Ara h 3. The deduced amino acid sequence of Ara h 3 shows homology to 11S seed-storage proteins. The recombinant form of this protein was expressed in a bacterial system and was recognized by serum IgE from approximately 45% of our peanut-allergic patient population. Serum IgE from these patients and overlapping, synthetic peptides were used to map the linear, IgE-binding epitopes of Ara h 3. Four epitopes, between 10 and 15 amino acids in length, were found within the primary sequence, with no obvious sequence motif shared by the peptides. One epitope is recognized by all Ara h 3-allergic patients. Mutational analysis of the epitopes revealed that single amino acid changes within these peptides could lead to a reduction or loss of IgE binding. By determining which amino acids are critical for IgE binding, it might be possible to alter the Ara h 3 **cDNA** to

encode a protein with a reduced IgE-binding capacity. These results will enable the design of improved diagnostic and therapeutic approaches for food-hypersensitivity reactions.

L30 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:154703 Document No.: PREV199800154703. Glycinin, a third major **peanut allergen** identified by soy-adsorbed serum IgE from peanut sensitive individuals. **Rabjohn, P.**; West, C. M.; Helm, E.; Helm, R.; Stanley, J. S.; Huang, S. K.; Sampson, H.; Burks, A. W.; Bannon, G. A.. Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S240. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Washington, DC, USA March 13-18, 1998 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L30 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2002 ACS
1998:141656 Characterization and epitope analysis of ARA h 3, a glycinin involved in peanut hypersensitivity.. Helm, Erica M.; **Rabjohn, Pat A.**; Stanley, J. Steven; West, C. Michael; Huang, S. K.; Sampson, H.; Burks, A. Wesley; Bannon, Gary A. (Department Chemistry, Hendrix College, Conway, AR, 72032, USA). Book of Abstracts, 215th ACS National Meeting, Dallas, March 29-April 2, CHED-179. American Chemical Society: Washington, D. C. (English) 1998. CODEN: 65QTAA.
AB Peanut allergy is a major health concern due to the severity of the allergic reaction, the lifelong persistence of the allergy, and the ubiquitous use of peanut as a protein supplement in processed foods. Using a previously unidentified **peanut allergen**, Ara h 3 **cDNA** clone was isolated, sequenced and found to be 1530 nucleotides and encoded a 510 amino acid protein. This sequence showed homol. to the glycinin family of seed storage proteins of common legumes. Synthetic peptides were used to det. which regions of the primary sequence served as linear B-cell epitopes for binding serum IgE from a population of peanut hypersensitivity patients. These epitopes were distributed evenly throughout the primary sequence and were six to ten amino acids in length. Further studies will be focused on identifying individual amino acids crit. for IgE binding. Once these amino acids are identified, it will be possible to mutate crit. residues to eliminate the ability of this protein to bind IgE.

L30 ANSWER 5 OF 6 MEDLINE
97330026 Document Number: 97330026. PubMed ID: 9186485. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major **peanut allergen** Ara h 2. Stanley J S; **King N**; Burks A W; Huang S K; Sampson H; Cockrell G; Helm R M; West C M; Bannon G A. (Department of Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1997 Jun 15) 342 (2) 244-53. Journal code: 6SK; 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.
AB A major **peanut allergen**, Ara h 2, is recognized by serum IgE from > 90% of patients with peanut hypersensitivity. Biochemical characterization of this allergen indicates that it is a glycoprotein of approximately 17.5 kDa. Using N-terminal amino acid sequence data from purified Ara h 2, oligonucleotide primers were synthesized and used to identify a clone (741 bp) from a peanut **cDNA** library. This clone was capable of encoding a 17.5-kDa protein with homology to the conglutin family of seed storage proteins. The major linear immunoglobulin E (IgE)-binding epitopes of this allergen were mapped using overlapping peptides synthesized on an activated cellulose membrane and pooled serum IgE from 15 peanut-sensitive patients. Ten IgE-binding epitopes were identified, distributed throughout the length of the Ara h 2 protein. Sixty-three percent of the amino acids represented in the epitopes were either polar uncharged or apolar residues. In an effort to determine

which, if any, of the 10 epitopes were recognized by the majority of patients with peanut hypersensitivity, each set of 10 peptides was probed individually with serum IgE from 10 different patients. All of the patient sera tested recognized multiple epitopes. Three epitopes (aa27-36, aa57-66, and aa65-74) were recognized by all patients tested. In addition, these three peptides bound more IgE than all the other epitopes combined, indicating that they are the immunodominant epitopes of the Ara h 2 protein. Mutational analysis of the Ara h 2 epitopes indicate that single amino acid changes result in loss of IgE binding. Two epitopes in region aa57-74 contained the amino acid sequence DPYSP that appears to be necessary for IgE binding. These results may allow for the design of improved diagnostic and therapeutic approaches to peanut hypersensitivity.

L30 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2002 ACS

1997:159532 Cloning of a portion of Ara h 3: A **peanut allergen**.. Helm, Erica M.; **Rabjohn, P. A.**; Burks, A. W.; Sampson, H. A.; Bannon, G. A. (Hendrix College, Conway, AR, 72032, USA). Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17, CHED-241. American Chemical Society: Washington, D. C. (English) 1997. CODEN: 64AOAA.

AB Four peanut proteins have been identified as major allergens in peanut hypersensitivity. This study involved the identification and sequencing of one of these proteins, Ara h 3. Sera from groups of allergic people were used to identify IgE binding proteins by Western blot anal. A .apprx. 14kD protein was isolated and a portion of it's amino acid sequence was used to derive oligonucleotide probes that were utilized as PCR primers and hybridization probes to clone the gene that encoded this protein. A **cDNA** clone carrying a 1200 bp insert was isolated. Northern blot anal. revealed that this insert hybridized to an .apprx.1.6 kb mRNA, indicating that the insert was not full length. DNA sequence anal. revealed that Ara h 3 was a glycinin, a seed storage protein. As a continuation of this study, the known sequence will be mutagenized to identify the amino acids that are important in causing peanut hypersensitivity.

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(FILE 'HOME' ENTERED AT 16:38:59 ON 13 FEB 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 16:39:21 ON 13 FEB 2002

L1	2	S NUCLEOTIDE AND MODIFIED ALLERGEN
L2	2	DUP REMOVE L1 (0 DUPLICATES REMOVED)
L3	36449	S POLYNUCLEOTIDE
L4	28	S L3 AND ALLERGEN
L5	1	S L4 AND MODIFIED
L6	28	DUP REMOVE L4 (0 DUPLICATES REMOVED)
L7	97315	S ALLERGEN
L8	2206	S L7 AND MODIFIED
L9	1	S L8 AND POLYNUCLEOTIDE
L10	469	S PEANUT ALLERGEN
L11	0	S L10 AND POLYNUCLEOTIDE
L12	0	S L10 AND REDUCE IGE BINDING
L13	15	S L10 AND ARA H1
L14	0	S L13 AND POLYNUCLEOTIDE
L15	8	DUP REMOVE L13 (7 DUPLICATES REMOVED)
L16	28	S ARA H2
L17	12	DUP REMOVE L16 (16 DUPLICATES REMOVED)
L18	12	S ARA H3
L19	7	DUP REMOVE L18 (5 DUPLICATES REMOVED)
L20	1901	S BANNON G?/AU OR BURKS W?/AU OR SAMPSON H?/AU OR SOSIN H?/AU
L21	130	S L20 AND PEANUT ALLERGEN
L22	80	DUP REMOVE L21 (50 DUPLICATES REMOVED)

L23 0 S L22 AND POLYNUCLEOTIDE
L24 11 S L22 AND CDNA
L25 11 DUP REMOVE L24 (0 DUPLICATES REMOVED)
L26 7752 S KING N?/AU OR MALEKI S?/AU OR CONNAUGHTON C?/AU OR KOPPER R?/
L27 93 S L26 AND PEANUT ALLERGEN
L28 50 DUP REMOVE L27 (43 DUPLICATES REMOVED)
L29 6 S L28 AND CDNA
L30 6 DUP REMOVE L29 (0 DUPLICATES REMOVED)

=> d l28 1-50 cbib abs

L28 ANSWER 1 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
2001:175395 Document No.: PREV200100175395. Allergenic and biophysical properties of purified **peanut allergens** before and after roasting. **Maleki, Soheila J. (1)**; Champagne, Elaine T. (1). (1) Southern Regional Research Center, USDA-ARS, New Orleans, LA USA. Journal of Allergy and Clinical Immunology, (February, 2001) Vol. 107, No. 2, pp. S188. print. Meeting Info.: 57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology New Orleans, Louisiana, USA March 16-21, 2001 ISSN: 0091-6749. Language: English. Summary Language: English.

L28 ANSWER 2 OF 50 MEDLINE DUPLICATE 2
2001262411 Document Number: 21203243. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major **peanut allergens** for use in immunotherapy. Bannon G A; Cockrell G; **Connaughton C**; West C M; Helm R; Stanley J S; **King N**; **Rabjohn P**; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major **peanut allergens**, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified **peanut allergens** were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. CONCLUSIONS: These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy. Copyright 2001 S. Karger AG, Basel

L28 ANSWER 3 OF 50 CAPLUS COPYRIGHT 2002 ACS
2000:628260 Document No. 133:221613 Site-specific mutated allergens for

decreased clinical reaction to allergy. Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A.; Sosin, Howard B.; **King, Nina E.**; **Maleki, Soheila J.**; **Connaughton, Cathie**; **Kopper, Randall A.**; **Rabjohn, Patrick A.**; **Shin, David S.**; Compadre, Cesar M. (The Board of Trustees of the University of Arkansas, USA; Mount Sinai School of Medicine of New York University). PCT Int. Appl. WO 2000052154 A2 20000908, 38 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US5487 20000302. PRIORITY: US 1999-PV122566 19990302; US 1999-PV122960 19990303; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been detd. that allergens, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be modified to be less allergenic by modifying the IgE-binding sites. The IgE binding sites can be converted to non-IgE binding sites by masking the site with a compd. that prevents IgE binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the IgE-binding epitope, to eliminate IgE binding. The method allows the protein to be altered as minimally as possible, other than within the IgE-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use **peanut allergens** to demonstrate alteration of IgE-binding sites. The crit. amino acids within each of the IgE-binding epitopes of the peanut protein that are important to Ig binding were detd. Substitution of even a single amino acid within each of the epitopes led to loss of IgE binding. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most crit. to IgE binding.

L28 ANSWER 4 OF 50 MEDLINE DUPLICATE 3

2000281673 Document Number: 20281673. PubMed ID: 10820263. Structure of the major **peanut allergen** Ara h 1 may protect IgE-binding epitopes from degradation. **Maleki S J**; **Kopper R A**; **Shin D S**; Park C W; Compadre C M; Sampson H; Burks A W; Bannon G A. (Departments ofPediatrics and Biochemistry and Molecular Biology and Biomedical Visualization Center, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, AR 72205, USA.) JOURNAL OF IMMUNOLOGY, (2000 Jun 1) 164 (11) 5844-9. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB In the past decade, there has been an increase in allergic reactions to peanut proteins, sometimes resulting in fatal anaphylaxis. The development of improved methods for diagnosis and treatment of peanut allergies requires a better understanding of the structure of the allergens. Ara h 1, a major **peanut allergen** belonging to the vicilin family of seed storage proteins, is recognized by serum IgE from >90% of peanut-allergic patients. In this communication, Ara h 1 was shown to form a highly stable homotrimer. Hydrophobic interactions were determined to be the main molecular force holding monomers together. A molecular model of the Ara h 1 trimer was constructed to view the stabilizing hydrophobic residues in the three dimensional structure. Hydrophobic amino acids that contribute to trimer formation are at the distal ends of the three dimensional structure where monomer-monomer contacts occur. Coincidentally, the majority of the IgE-binding epitopes are also located in this region, suggesting that they may be protected from digestion by the monomer-monomer contacts. On incubation of Ara h 1 with digestive enzymes, various protease-resistant fragments containing IgE-binding sites

were identified. The highly stable nature of the Ara h 1 trimer, the presence of digestion resistant fragments, and the strategic location of the IgE-binding epitopes indicate that the quaternary structure of a protein may play a significant role in overall allergenicity.

L28 ANSWER 5 OF 50 CAPLUS COPYRIGHT 2002 ACS
2000:789724 Document No. 134:339830 The effects of roasting on the allergenic properties of peanut proteins. **Maleki, Soheila J.**; Chung, Si-Yin; Champagne, Elaine T.; Raufman, Jean-Pierre (ARS-Southern Regional Research Center, USDA, New Orleans, LA, 70124, USA). J. Allergy Clin. Immunol., 106(4), 763-768 (English) 2000. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..
AB Because of the widespread use of peanut products, peanut allergenicity is a major health concern in the United States. The effect or effects of thermal processing (roasting) on the allergenic properties of peanut proteins have rarely been addressed. We sought to assess the biochem. effects of roasting on the allergenic properties of peanut proteins. Competitive inhibition ELISA was used to compare the IgE-binding properties of roasted and raw peanut exts. A well-characterized in vitro model was used to test whether the Maillard reaction contributes to the allergenic properties of peanut proteins. The allergic properties were measured by using ELISA, digestion by gastric secretions, and stability of the proteins to heat and degrdn. Here we report that roasted peanuts from two different sources bound IgE from patients with peanut allergy at approx. 90-fold higher levels than the raw peanuts from the same peanut cultivars. The purified major allergens Ara h 1 and Ara h 2 were subjected to the Maillard reaction in vitro and compared with corresponding unreacted samples for allergenic properties. Ara h 1 and Ara h 2 bound higher levels of IgE and were more resistant to heat and digestion by gastrointestinal enzymes once they had undergone the Maillard reaction. The data presented here indicate that thermal processing may play an important role in enhancing the allergenic properties of peanuts and that the protein modifications made by the Maillard reaction contribute to this effect.

L28 ANSWER 6 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2000:140432 Document No.: PREV200000140432. Development of hypoallergenic proteins for use in treating patients with peanut hypersensitivity. Burks, A. W.; **King, N.**; **Rabjohn, P.**; Buzen, F.; West, C. M.; Helm, R.; Stanley, J. S.; Sampson, H.; Bannon, G. A.. Journal of Allergy and Clinical Immunology., (Jan., 2000) Vol. 105, No. 1 part 2, pp. S310. Meeting Info.: 56th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. San Diego, California, USA March 03-08, 2000 American Academy of Allergy, Asthma and Immunology. ISSN: 0091-6749. Language: English. Summary Language: English.

L28 ANSWER 7 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2000:168036 Document No.: PREV200000168036. Development of a safe immunotherapeutic strategy for treating patients with peanut hypersensitivity. Burks, A. Wesley (1); **King, Nina**; **Connaughton, Cathie**; **Rabjohn, Pat**; Buzen, Fred; West, C. Michael; Helm, Ricki; Stanley, J. Steven; Sampson, Hugh A.; Bannon, Gary A.. (1) Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR USA. Pediatric Research., (April, 2000) Vol. 47, No. 4 Part 2, pp. 15A. Meeting Info.: Joint Meeting of the Pediatric Academic Societies and the American Academy of Pediatrics. Boston, Massachusetts, USA May 12-16, 2000 American Academy of Pediatrics. ISSN: 0031-3998. Language: English. Summary Language: English.

L28 ANSWER 8 OF 50 CAPLUS COPYRIGHT 2002 ACS
2000:174404 Document No. 132:193205 Molecular cloning, epitope analysis, and mutagenesis of the **peanut allergen**, Ara h 3. **Rabjohn, Patrick Allan** (Univ. of Arkansas for Medical Sciences, Little Rock, AR, USA). 136 pp. Avail. UMI, Order No. DA9945174 From:

Diss. Abstr. Int., B 2000, 60(8), 3753 (English) 1999.
AB Unavailable

L28 ANSWER 9 OF 50 CAPLUS COPYRIGHT 2002 ACS
2000:174402 Document No. 132:206870 Modulation of the allergenicity of a major **peanut allergen** Ara h 2 by site-directed mutagenesis of its IgE binding epitopes. **King, Nina Evgenievna** (Univ. of Arkansas for Medical Sciences, Little Rock, AR, USA). 125 pp. Avail. UMI, Order No. DA9945176 From: Diss. Abstr. Int., B 2000, 60(8), 3746 (English) 1999.
AB Unavailable

L28 ANSWER 10 OF 50 CAPLUS COPYRIGHT 2002 ACS
1999:594994 Document No. 131:227660 Tertiary structure of **peanut allergen** Ara h 1. Burks, Wesley, Jr.; Helm, Ricki M.; Cockrell, Gael; Bannon, Gary A.; Stanley, J. Steven; **Shin, David S.**; Sampson, Hugh; Compadre, Cesar M.; Huang, Shau K. (Board of Trustees of the University of Arkansas, USA). PCT Int. Appl. WO 9945961 A1 19990916, 193 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US5494 19990312. PRIORITY: US 1998-PV77763 19980312.
AB Ara h 1, a major **peanut allergen**, has been isolated and shown to contain 23 linear IgE-binding epitopes, 6-10 residues in length. Anal. of wild-type and mutant peptides with single amino acids substitutions showed that amino acids residing in the middle of the epitope were more crit. for IgE binding; that polar charged residues occurred more frequently within the epitope while apolar residues were more important for IgE binding; and that a single amino acid substitution in an epitope resulted in a loss of ability to bind IgE. In addn., a homol.-based mol. model of the Ara h 1 protein representing residues 171-586 was made and allowed visualization of epitopes 10-22. The majority of these epitopes appear clustered and many of the crit. amino acids involved in binding are evenly distributed on the surface. The information from the mutational anal. and the mol. model will aid in the design of immunotherapies.

L28 ANSWER 11 OF 50 MEDLINE DUPLICATE 4
1999146968 Document Number: 99146968. PubMed ID: 10021462. Molecular cloning and epitope analysis of the **peanut allergen** Ara h 3. **Rabjohn P**; Helm E M; Stanley J S; West C M; Sampson H A; Burks A W; Bannon G A. (Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) JOURNAL OF CLINICAL INVESTIGATION, (1999 Feb) 103 (4) 535-42. Journal code: HS7; 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB Peanut allergy is a significant IgE-mediated health problem because of the increased prevalence, potential severity, and chronicity of the reaction. Following our characterization of the two **peanut allergens** Ara h 1 and Ara h 2, we have isolated a cDNA clone encoding a third **peanut allergen**, Ara h 3. The deduced amino acid sequence of Ara h 3 shows homology to 11S seed-storage proteins. The recombinant form of this protein was expressed in a bacterial system and was recognized by serum IgE from approximately 45% of our peanut-allergic patient population. Serum IgE from these patients and overlapping, synthetic peptides were used to map the linear, IgE-binding epitopes of Ara h 3. Four epitopes, between 10 and 15 amino acids in length, were found within the primary sequence, with no obvious sequence motif shared by the peptides. One epitope is recognized by all Ara h

3-allergic patients. Mutational analysis of the epitopes revealed that single amino acid changes within these peptides could lead to a reduction or loss of IgE binding. By determining which amino acids are critical for IgE binding, it might be possible to alter the Ara h 3 cDNA to encode a protein with a reduced IgE-binding capacity. These results will enable the design of improved diagnostic and therapeutic approaches for food-hypersensitivity reactions.

L28 ANSWER 12 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:143127 The Genuine Article (R) Number: 165FC. T-cell responses to **peanut allergens**: Potential for development of novel immunotherapeutic strategies for food allergic disease.. **Maleki S J (Reprint)**; **Connaughton C**; **Kopper R A**; Sampson H A; Bannon G A; Burks A W. USDA, WASHINGTON, DC 20250; ARKANSAS CHILDRENS HOSP, LITTLE ROCK, AR 72202; UNIV ARKANSAS MED SCI, LITTLE ROCK, AR 72205; MT SINAI SCH MED, NEW YORK, NY 10029. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1999) Vol. 103, No. 1, Part 2, Supp. [S], pp. 415-415. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 13 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:143099 The Genuine Article (R) Number: 165FC. Mutational analysis of the IgE-binding epitopes of the **peanut allergen**, Ara h 3: a member of the glycinin family of seed-storage proteins.. **Rabjohn P (Reprint)**; Burks A W; Sampson H A; Bannon G A. UNIV ARKANSAS MED SCI, LITTLE ROCK, AR 72205; MT SINAI SCH MED, NEW YORK, NY 10029. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1999) Vol. 103, No. 1, Part 2, Supp. [S], pp. 387-387. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA . Language: English.

L28 ANSWER 14 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:143089 The Genuine Article (R) Number: 165FC. Modulation of the reactivity of the major **peanut allergen** Ara h 1 through epitope characterization, structural analysis, and mutation.. **Shin D S (Reprint)**; Compadre C M; Sampson H A; Burks A W; Bannon G A. UNIV ARKANSAS MED SCI, DEPT PEDIAT, DEPT BIOCHEM & MOL BIOL, BIOMED VISUALIZAT CTR, LITTLE ROCK, AR 72205; MT SINAI SCH MED, DEPT PEDIAT, NEW YORK, NY 10069. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1999) Vol. 103, No. 1, Part 2, Supp. [S], pp. 376-376. Publisher: MOSBY-YEAR BOOK INC . 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 15 OF 50 MEDLINE DUPLICATE 5
1999242481 Document Number: 99242481. PubMed ID: 10224426. Tertiary structure and biophysical properties of a major **peanut allergen**, implications for the production of a hypoallergenic protein. Bannon G A; **Shin D**; **Maleki S**; **Kopper R**; Burks A W. (Departments of Biochemistry and Molecular Biology and Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR, USA.. Bannongarya@exchange.uams.edu) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1999 Feb-Apr) 118 (2-4) 315-6. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

L28 ANSWER 16 OF 50 MEDLINE DUPLICATE 6
1999242480 Document Number: 99242480. PubMed ID: 10224425. Modification of a major **peanut allergen** leads to loss of IgE binding. Burks A W; **King N**; Bannon G A. (Departments of Pediatrics and Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR, USA.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1999 Feb-Apr) 118 (2-4) 313-4. Journal code: BJ7; 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

L28 ANSWER 17 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:142972 The Genuine Article (R) Number: 165FC. Modulation of the allergenicity of a major **peanut allergen**, Ara h 2 by mutagenesis of its immunodominant IgE binding epitopes.. **King N (Reprint)**; **Maleki S J**; Sampson H; Burks A W; Bannon G A. UNIV ARKANSAS MED SCI, LITTLE ROCK, AR 72205; USDA, WASHINGTON, DC; MT SINAI SCH MED, NEW YORK, NY 10029. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1999) Vol. 103, No. 1, Part 2, Supp. [S], pp. 258-258. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 18 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:142971 The Genuine Article (R) Number: 165FC. The effect of digestion on the allergenicity of the major **peanut allergen** Ara h 1.. **Kopper R A (Reprint)**; **Maleki S**; Sampson H A; Burks A W; Bannon G A. UNIV ARKANSAS MED SCI, LITTLE ROCK, AR 72205; MT SINAI SCH MED, NEW YORK, NY 10029. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1999) Vol. 103, No. 1, Part 2, Supp. [S], pp. 257-257. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 19 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:125710 Document No.: PREV199900125710. T-cell responses to **peanut allergens**: Potential for development of novel immunotherapeutic strategies for food allergic disease. **Maleki, S. J. (1)**; **Connaughton, C.**; **Kopper, R. A.**; Sampson, H. A.; Bannon, G. A.; Burks, A. W.. (1) USDA, Fayetteville, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1999) Vol. 103, No. 1 PART 2, pp. S109. Meeting Info.: 55th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Orlando, Florida, USA February 26-March 3, 1999 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 20 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:153040 Document No.: PREV199900153040. Mutational analysis of the IgE-binding epitopes of the **peanut allergen**, Ara h 3: A member of the glycinin family of seed-storage proteins. **Rabjohn, P. (1)**; Burks, A. W. (1); Sampson, H. A.; Bannon, G. A. (1). (1) Univ. Arkansas Med. Sci., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1999) Vol. 103, No. 1 PART 2, pp. S101. Meeting Info.: 55th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Orlando, Florida, USA February 26-March 3, 1999 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 21 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:158132 Document No.: PREV199900158132. Modulation of the reactivity of the major **peanut allergen** Ara h 1 through epitope characterization, structural analysis, and mutation. **Shin, D. S. (1)**; Compadre, C. M.; Sampson, H. A.; Burks, A. W.; Bannon, G. A.. (1) Dep. Biochem. Mol. Biol., Biomed. Visualization Cent., Univ. Arkansas Med. Sci., Little Rock, AR 72205 USA. Journal of Allergy and Clinical Immunology, (Jan., 1999) Vol. 103, No. 1 PART 2, pp. S99. Meeting Info.: 55th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Orlando, Florida, USA February 26-March 3, 1999 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 22 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:134462 Document No.: PREV199900134462. Modulation of the allergenicity of a major **peanut allergen**, Ara h 2 by mutagenesis of its immunodominant IgE binding epitopes. **King, N. (1)**; **Maleki, S. J.**; Sampson, H.; Burks, A. W. (1); Bannon, G. A. (1). (1) Univ.

Arkansas Med. Sci., Little Rock, AR 72201 USA. Journal of Allergy and Clinical Immunology, (Jan., 1999) Vol. 103, No. 1 PART 2, pp. S67. Meeting Info.: 55th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Orlando, Florida, USA February 26-March 3, 1999 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 23 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:136286 Document No.: PREV199900136286. The effect of digestion on the allergenicity of the major **peanut allergen** Ara h 1. **Kopper, R. A. (1)**; **Maleki, S.**; Sampson, H. A.; Burks, A. W. (1); Bannon, G. A. (1). (1) Univ. Arkansas Med. Sci., Little Rock, AR 72201 USA. Journal of Allergy and Clinical Immunology, (Jan., 1999) Vol. 103, No. 1 PART 2, pp. S67. Meeting Info.: 55th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Orlando, Florida, USA February 26-March 3, 1999 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 24 OF 50 CAPLUS COPYRIGHT 2002 ACS
1998:383080 Document No. 129:121594 Biochemical and structural analysis of the IgE binding sites on Ara h1, an abundant and highly allergenic peanut protein. **Shin, David S.**; Compadre, Cesar M.; **Maleki, Soheila J.**; **Kopper, Randall A.**; Sampson, Hugh; Huang, Shau K.; Burks, A. Wesley; Bannon, Gary A. (Department of Biochemistry & Molecular Biology, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA). J. Biol. Chem., 273(22), 13753-13759 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB Allergy to peanut is a significant IgE-mediated health problem because of the high prevalence, potential severity, and chronicity of the reaction. Ara h1, an abundant peanut protein, is recognized by serum IgE from >90% of peanut-sensitive individuals. It has been shown to belong to the vicilin family of seed storage proteins and to contain 23 linear IgE binding epitopes. Here, the authors detd. the crit. amino acids within each of the IgE binding epitopes of Ara h1 that are important for Ig binding. Surprisingly, substitution of a single amino acid within each of the epitopes led to loss of IgE binding. In addn., hydrophobic residues appeared to be most crit. for IgE binding. The position of each of the IgE binding epitopes on a homol.-based mol. model of Ara h1 showed that they were clustered into 2 main regions, despite their more even distribution in the primary sequence. Finally, the authors have shown that Ara h1 forms a stable trimer by the use of a reproducible fluorescence assay. This information will be important in studies designed to reduce the risk of peanut-induced anaphylaxis by lowering the IgE binding capacity of the allergen.

L28 ANSWER 25 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:176822 The Genuine Article (R) Number: YW339. Glycinin, a third major **peanut allergen** identified by soy-adsorbed serum IgE from peanut sensitive individuals.. **Rabjohn P (Reprint)**; West C M; Helm E; Helm R; Stanley J S; Huang S K; Sampson H; Burks A W; Bannon G A. UNIV ARKANSAS, SCH MED, LITTLE ROCK, AR 72204; JOHNS HOPKINS UNIV, BALTIMORE, MD; MT SINAI HOSP, NEW YORK, NY 10029. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1998) Vol. 101, No. 1, Part 2, pp. 996-996. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 26 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:176820 The Genuine Article (R) Number: YW339. Rapid isolation of **peanut allergens** and their physical chemical and biological characterization.. **Kopper R (Reprint)**; **Maleki S**; Helm R; Sampson H; Huang S K; Cockrell G; Burks A W; Bannon G A. UNIV ARKANSAS, SCH MED, LITTLE ROCK, AR 72204; JOHNS HOPKINS UNIV, BALTIMORE, MD; MT SINAI HOSP, NEW YORK, NY 10029. JOURNAL OF ALLERGY AND

CLINICAL IMMUNOLOGY (JAN 1998) Vol. 101, No. 1, Part 2, pp. 994-994. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 27 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:176439 The Genuine Article (R) Number: YW339. T-cell responses in food allergy: Identification of T-cell epitopes on a major **peanut allergen**.. **Maleki S (Reprint)**; Wang Q F; **Connaughton C**; Cockrell G; Helm R; Huang S K; Sampson H; Bannon G A; Burks A W. UNIV ARKANSAS, SCH MED, LITTLE ROCK, AR 72204; MT SINAI HOSP, NEW YORK, NY 10029; JOHNS HOPKINS UNIV, BALTIMORE, MD. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1998) Vol. 101, No. 1, Part 2, pp. 609-609. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 28 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:176209 The Genuine Article (R) Number: YW339. Tertiary structure of the major **peanut allergen** Ara h 1: Implications for the bioengineering of a hypoallergenic protein.. **Shin D (Reprint)**; Sampson H; Helm R; Huang S K; Burks A W; Bannon G A. UNIV ARKANSAS, SCH MED, LITTLE ROCK, AR 72204; JOHNS HOPKINS UNIV, BALTIMORE, MD; MT SINAI HOSP, NEW YORK, NY 10029. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1998) Vol. 101, No. 1, Part 2, pp. 379-379. Publisher: MOSBY-YEAR BOOK INC . 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 29 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:154701 Document No.: PREV199800154701. Rapid isolation of **peanut allergens** and their physical chemical and biological characterization. **Kopper, R. (1)**; **Maleki, S.**; Helm, R.; Sampson, H.; Huang, S. K.; Cockrell, G.; Burks, A. W.; Bannon, G. A.. (1) Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S240. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Washington, DC, USA March 13-18, 1998 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 30 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:154703 Document No.: PREV199800154703. Glycinin, a third major **peanut allergen** identified by soy-adsorbed serum IgE from peanut sensitive individuals. **Rabjohn, P.**; West, C. M.; Helm, E.; Helm, R.; Stanley, J. S.; Huang, S. K.; Sampson, H.; Burks, A. W.; Bannon, G. A.. Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S240. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Washington, DC, USA March 13-18, 1998 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 31 OF 50 CAPLUS COPYRIGHT 2002 ACS
1998:141656 Characterization and epitope analysis of ARA h 3, a glycinin involved in peanut hypersensitivity.. Helm, Erica M.; **Rabjohn, Pat A.**; Stanley, J. Steven; West, C. Michael; Huang, S. K.; Sampson, H.; Burks, A. Wesley; Bannon, Gary A. (Department Chemistry, Hendrix College, Conway, AR, 72032, USA). Book of Abstracts, 215th ACS National Meeting, Dallas, March 29-April 2, CHED-179. American Chemical Society: Washington, D. C. (English) 1998. CODEN: 65QTAA.
AB Peanut allergy is a major health concern due to the severity of the allergic reaction, the lifelong persistence of the allergy, and the ubiquitous use of peanut as a protein supplement in processed foods. Using a previously unidentified **peanut allergen**, Ara h 3 cDNA clone was isolated, sequenced and found to be 1530 nucleotides and

encoded a 510 amino acid protein. This sequence showed homol. to the glycinin family of seed storage proteins of common legumes. Synthetic peptides were used to det. which regions of the primary sequence served as linear B-cell epitopes for binding serum IgE from a population of peanut hypersensitivity patients. These epitopes were distributed evenly throughout the primary sequence and were six to ten amino acids in length. Further studies will be focused on identifying individual amino acids crit. for IgE binding. Once these amino acids are identified, it will be possible to mutate crit. residues to eliminate the ability of this protein to bind IgE.

L28 ANSWER 32 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:154319 Document No.: PREV199800154319. T-cell responses in food allergy: Identification of T-cell epitopes on a major **peanut allergen**. **Maleki, S. (1)**; Wang, Q. F.; **Connaughton, C.**; Cockrell, G.; Helm, R.; Huang, S. K.; Sampson, H.; Bannon, G. A.; Burks, A. W.. (1) Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S147. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Washington, DC, USA March 13-18, 1998 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 33 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:154089 Document No.: PREV199800154089. Tertiary structure of the major **peanut allergen** Ara h 1: Implications for the bioengineering of a hypoallergenic protein. **Shin, D. (1)**; Sampson, H.; Helm, R.; Huang, S. K.; Burks, A. W.; Bannon, G. A.. (1) Univ. Ark. Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S90. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Washington, DC, USA March 13-18, 1998 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L28 ANSWER 34 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
97:165889 The Genuine Article (R) Number: WH142. Characterization of a major **peanut allergen**: Mutational analysis of the Ara h 1 IgE binding epitopes.. **Shin D (Reprint)**; Sampson H A; Huang S K; Compadre C; Burks A W; Bannon G A. UNIV ARKANSAS, SCH MED, LITTLE ROCK, AR 72204; JOHNS HOPKINS UNIV, BALTIMORE, MD. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1997) Vol. 99, No. 1, Part 2, Supp. [S], pp. 570-570. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L28 ANSWER 35 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
97:165888 The Genuine Article (R) Number: WH142. Cloning, epitope mapping, and mutual analysis of Ara h 2, a major **peanut allergen** .. Burks A W (Reprint); **King N**; West C M; Stanley J S; Cockrell G; Helm R; Huang S K; Sampson H A; Bannon G A. UNIV ARKANSAS, SCH MED, LITTLE ROCK, AR 72204; JOHNS HOPKINS UNIV, BALTIMORE, MD. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1997) Vol. 99, No. 1, Part 2, Supp. [S], pp. 569-569. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA . Language: English.

L28 ANSWER 36 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
97:165887 The Genuine Article (R) Number: WH142. Ara h 3, a **peanut allergen** identified by using peanut sensitive patient sera absorbed with soy proteins.. Bannon G A (Reprint); Li X F; **Rabjohn P**; Stanley J S; Burks A W; Huang S K; Sampson H A. UNIV ARKANSAS, SCH MED, LITTLE ROCK, AR 72204; JOHNS HOPKINS UNIV, BALTIMORE, MD. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1997) Vol. 99, No. 1, Part 2, Supp. [S], pp. 568-568. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA

. Language: English.

L28 ANSWER 37 OF 50 MEDLINE DUPLICATE 7
97296397 Document Number: 97296397. PubMed ID: 9151961. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. Burks A W; **Shin D**; Cockrell G; Stanley J S; Helm R M; Bannon G A. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) EUROPEAN JOURNAL OF BIOCHEMISTRY, (1997 Apr 15) 245 (2) 334-9. Journal code: EMZ; 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Peanut allergy is a significant health problem because of the prevelance and potential severity of the allergic reaction. Serum IgE from patients with documented peanut hypersensitivity reactions and overlapping peptides were used to identify the IgE-binding epitopes on the major **peanut allergen**, Ara h 1. At least twenty-three different linear IgE-binding epitopes, located throughout the length of the Ara h 1 protein, were identified. All of the epitopes were 6-10 amino acids in length, but there was no obvious sequence motif shared by all peptides. Four of the peptides appeared to be immunodominant IgE-binding epitopes in that they were recognized by serum from more than 80% of the patients tested and bound more IgE than any of the other Ara h 1 epitopes. Mutational analysis of the immunodominant epitopes revealed that single amino acid changes within these peptides had dramatic effects on IgE-binding characteristics. The identification and determination of the IgE-binding capabilities of core amino acids in epitopes on the Ara h 1 protein will make it possible to address the pathophysiologic and immunologic mechanisms regarding peanut hypersensitivity reactions specifically and food hypersensitivity in general.

L28 ANSWER 38 OF 50 MEDLINE DUPLICATE 8
97330026 Document Number: 97330026. PubMed ID: 9186485. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major **peanut allergen** Ara h 2. Stanley J S; **King N**; Burks A W; Huang S K; Sampson H; Cockrell G; Helm R M; West C M; Bannon G A. (Department of Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1997 Jun 15) 342 (2) 244-53. Journal code: 6SK; 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB A major **peanut allergen**, Ara h 2, is recognized by serum IgE from > 90% of patients with peanut hypersensitivity. Biochemical characterization of this allergen indicates that it is a glycoprotein of approximately 17.5 kDa. Using N-terminal amino acid sequence data from purified Ara h 2, oligonucleotide primers were synthesized and used to identify a clone (741 bp) from a peanut cDNA library. This clone was capable of encoding a 17.5-kDa protein with homology to the conglutin family of seed storage proteins. The major linear immunoglobulin E (IgE)-binding epitopes of this allergen were mapped using overlapping peptides synthesized on an activated cellulose membrane and pooled serum IgE from 15 peanut-sensitive patients. Ten IgE-binding epitopes were identified, distributed throughout the length of the Ara h 2 protein. Sixty-three percent of the amino acids represented in the epitopes were either polar uncharged or apolar residues. In an effort to determine which, if any, of the 10 epitopes were recognized by the majority of patients with peanut hypersensitivity, each set of 10 peptides was probed individually with serum IgE from 10 different patients. All of the patient sera tested recognized multiple epitopes. Three epitopes (aa27-36, aa57-66, and aa65-74) were recognized by all patients tested. In addition, these three peptides bound more IgE than all the other epitopes combined, indicating that they are the immunodominant epitopes of the Ara h 2 protein. Mutational analysis of the Ara h 2 epitopes indicate that single amino acid changes result in loss of IgE binding. Two epitopes in region

aa57-74 contained the amino acid sequence DPYSP that appears to be necessary for IgE binding. These results may allow for the design of improved diagnostic and therapeutic approaches to peanut hypersensitivity.

L28 ANSWER 39 OF 50 CAPLUS COPYRIGHT 2002 ACS
1997:159532 Cloning of a portion of Ara h 3: A **peanut allergen**.. Helm, Erica M.; **Rabjohn, P. A.**; Burks, A. W.; Sampson, H. A.; Bannon, G. A. (Hendrix College, Conway, AR, 72032, USA). Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17, CHED-241. American Chemical Society: Washington, D. C. (English) 1997. CODEN: 64AOAA.

AB Four peanut proteins have been identified as major allergens in peanut hypersensitivity. This study involved the identification and sequencing of one of these proteins, Ara h 3. Sera from groups of allergic people were used to identify IgE binding proteins by Western blot anal. A .apprx. 14kD protein was isolated and a portion of it's amino acid sequence was used to derive oligonucleotide probes that were utilized as PCR primers and hybridization probes to clone the gene that encoded this protein. A cDNA clone carrying a 1200 bp insert was isolated. Northern blot anal. revealed that this insert hybridized to an .apprx.1.6 kb mRNA, indicating that the insert was not full length. DNA sequence anal. revealed that Ara h 3 was a glycinin, a seed storage protein. As a continuation of this study, the known sequence will be mutagenized to identify the amino acids that are important in causing peanut hypersensitivity.

L28 ANSWER 40 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:144089 Document No.: PREV199799443292. Characterization of a major **peanut allergen**: Mutational analysis of the Ara h 1 IgE binding epitopes. **Shin, D.**; Sampson, H. A.; Huang, S. K.; Compadre, C.; Burkds, A. W.; Bannon, G. A.. Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (1997) Vol. 99, No. 1 PART 2, pp. S141. Meeting Info.: Joint Meeting of the American Academy of Allergy, Asthma and Immunology, the American Association of Immunologists and the Clinical Immunology Society San Francisco, California, USA February 21-26, 1997 ISSN: 0091-6749. Language: English.

L28 ANSWER 41 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:144088 Document No.: PREV199799443291. Cloning, epitope mapping, and mutational analysis of Ara h 2, a major **peanut allergen** . Burks, A. W.; **King, N.**; West, C. M.; Stanley, J. S.; Cockrell, G.; Helm, R.; Huang, S. K.; Sampson, H. A.; Bannon, G. A.. Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (1997) Vol. 99, No. 1 PART 2, pp. S141. Meeting Info.: Joint Meeting of the American Academy of Allergy, Asthma and Immunology, the American Association of Immunologists and the Clinical Immunology Society San Francisco, California, USA February 21-26, 1997 ISSN: 0091-6749. Language: English.

L28 ANSWER 42 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:144087 Document No.: PREV199799443290. Ara h 3, a **peanut allergen** identified by using peanut sensitive patient sera absorbed with soy proteins. Bannon, G. A.; Li, X.-F.; **Rabjohn, P.** ; Stanley, J. S.; Burks, A. W.; Huang, S. K.; Sampson, H. A.. Univ. Arkansas Med. Sch., Little Rock, AR USA. Journal of Allergy and Clinical Immunology, (1997) Vol. 99, No. 1 PART 2, pp. S141. Meeting Info.: Joint Meeting of the American Academy of Allergy, Asthma and Immunology, the American Association of Immunologists and the Clinical Immunology Society San Francisco, California, USA February 21-26, 1997 ISSN: 0091-6749. Language: English.

L28 ANSWER 43 OF 50 MEDLINE DUPLICATE 9
95155712 Document Number: 95155712. PubMed ID: 7531731. Epitope

specificity of the major **peanut allergen**, Ara h II. Burks A W; Cockrell G; **Connaughton C**; Karpas A; Helm R M. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital, Little Rock 72202.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1995 Feb) 95 (2) 607-11. Journal code: H53; 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB The antigenic and allergenic structure of Ara h II, a major allergen of peanuts, was investigated with the use of four monoclonal antibodies obtained from BALB/c mice immunized with purified Ara h II. Our previous studies with monoclonal antibodies generated to **peanut allergens** showed this method to be useful for epitope mapping. When used as a solid phase in an ELISA, these monoclonal antibodies captured peanut antigen, which bound human IgE from patients with positive peanut challenge responses. The Ara h II monoclonal antibodies were found to be specific for peanut antigens when binding for other legumes was examined. In ELISA inhibition studies with the monoclonal antibodies, we identified two different antigenic sites on Ara h II. In similar studies with pooled human IgE serum from patients with positive challenge responses to peanuts, we identified two closely related IgE-binding epitopes. These characterized monoclonal antibodies to Ara h II will be useful for future studies to immunoaffinity purify the Ara h II allergen and to use in conjunction with recombinant technology for determining structure-function relationships.

L28 ANSWER 44 OF 50 MEDLINE DUPLICATE 10

94216650 Document Number: 94216650. PubMed ID: 7513004. Epitope specificity and immunoaffinity purification of the major **peanut allergen**, Ara h I. Burks A W; Cockrell G; **Connaughton C**; Helm R M. (Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1994 Apr) 93 (4) 743-50. Journal code: H53; 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB The antigenic and allergenic structure of Ara h I, a major allergen of peanuts, was investigated with the use of seven monoclonal antibodies obtained from BALB/c mice immunized with purified Ara h I. Previous work with monoclonal antibodies produced to allergens has primarily been done with inhalant allergens. Only recently have the major allergens of various foods been determined so that investigations with monoclonal antibodies into the allergenic epitopes could begin. When used as a solid phase in an ELISA, these monoclonal antibodies captured peanut antigen, which bound human IgE from patients with positive results to challenges to peanuts. The Ara h I monoclonal antibodies were found to be specific for peanut antigens when binding for other legumes was examined. In ELISA inhibition studies with the monoclonal antibodies, we identified four different antigenic sites on Ara h I. In related studies with pooled human IgE serum from patients with positive results to challenges to peanuts, we identified three similar IgE-binding epitopes. As a means of purifying the Ara h I allergen, we prepared an immunoaffinity column with monoclonal antibody 8D9. We eluted from this column the allergen Ara h I, which had a mean molecular weight of 63.5 kd and which bound human IgE from individual and pooled serum of patients with peanut sensitivity.

L28 ANSWER 45 OF 50 MEDLINE DUPLICATE 11

93094482 Document Number: 93094482. PubMed ID: 1460200. Identification and characterization of a second major **peanut allergen**, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. Burks A W; Williams L W; **Connaughton C**; Cockrell G; O'Brien T J; Helm R M. (University of Arkansas for Medical Sciences, Little Rock.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1992 Dec) 90 (6 Pt 1) 962-9. Journal code: H53; 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB Peanuts are frequently a cause of food hypersensitivity reactions in children. Serum from nine patients with atopic dermatitis and a positive double-blind, placebo-controlled, food challenge to peanut were used in

the process of identification and purification of the **peanut allergens**. Identification of a second major **peanut allergen** was accomplished with use of various biochemical and molecular techniques. Anion exchange chromatography of the crude peanut extract produced several fractions that bound IgE from the serum of the patient pool with positive challenges. By measuring antipeanut specific IgE and by IgE-specific immunoblotting we have identified an allergic component that has two closely migrating bands with a mean molecular weight of 17 kd. Two-dimensional gel electrophoresis of this fraction revealed it to have a mean isoelectric point of 5.2. According to allergen nomenclature of the IUIS Subcommittee for Allergen Nomenclature this allergen is designated, Ara h II (Arachis hypogaea).

L28 ANSWER 46 OF 50 SCISEARCH COPYRIGHT 2002 ISI (R)
93:8475 The Genuine Article (R) Number: KD190. ALLERGENICITY OF PEANUT AND SOYBEAN EXTRACTS ALTERED BY CHEMICAL OR THERMAL-DENATURATION IN PATIENTS WITH ATOPIC-DERMATITIS AND POSITIVE FOOD CHALLENGES. BURKS A W (Reprint); WILLIAMS L W; THRESHER W; **CONNAUGHTON C**; COCKRELL G; HELM R M. ARKANSAS CHILDRENS HOSP, 800 MARSHALL ST, LITTLE ROCK, AR, 72202 (Reprint); CENT SOYA, FT WAYNE, IN, 00000; UNIV ARKANSAS MED SCI HOSP, DEPT PEDIAT, DIV PEDIAT IMMUNOL & ALLERGY, LITTLE ROCK, AR, 72205. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (DEC 1992) Vol. 90, No. 6, Part 1, pp. 889-897. ISSN: 0091-6749. Pub. country: USA. Language: ENGLISH. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Peanuts and soybeans are two of the six most common foods to cause food hypersensitivity reactions in children. We used the serum of 10 patients with atopic dermatitis and positive double-blind, placebo-controlled, food challenges to peanut and two patients with atopic dermatitis and positive double-blind, placebo-controlled, food challenges to soybean to investigate the change in IgE-specific and IgG-specific bending to these proteins altered by either chemical or thermal denaturation. We used IgE- and IgG-specific ELISA-inhibition analyses to compare these effects on the crude peanut and crude soy extracts, as well as on the major allergenic fractions of both proteins. Heating the soy proteins at various temperatures and time intervals did not significantly change the IgE- or IgG-specific binding of the soy positive pooled serum. When the peanut proteins were subjected to similar heating experiments, the IgE- and IgG-specific binding did not change. When these same proteins were treated with enzymes in the immobilized digestive enzyme assay system used to mimic human digestion, the binding of IgE to the crude peanut and crude soy extracts was reduced; 100-fold for peanut and 10-fold for soybean. Therefore it appears that thermal denaturation of peanut and soybean protein extracts does not enhance or reduce IgE- and IgG-specific binding activity. Chemical denaturation appears to minimally reduce the binding of these proteins.

L28 ANSWER 47 OF 50 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
1992:203434 Document No.: BR42:96509. MONOCLONAL ANTIBODY ENZYME-LINKED IMMUNOSORBENT ASSAY ELISA FOR ARA H I A MAJOR **PEANUT ALLERGEN**. COCKRELL G; **CONNAUGHTON C**; HELM R M; BURKS A W. LITTLE ROCK, ARKANSAS.. FORTY-EIGHTH ANNUAL MEETING OF THE AMERICAN ACADEMY OF ALLERGY AND IMMUNOLOGY, ORLANDO, FLORIDA, USA, MARCH 6-11, 1992. J ALLERGY CLIN IMMUNOL. (1992) 89 (1 PART 2), 298. CODEN: JACIBY. ISSN: 0091-6749. Language: English.

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1992:203435 Document No.: BR42:96510. THE IDENTIFICATION OF PEANUT AGGLUTININ AS A **PEANUT ALLERGEN** IN PATIENTS WITH ATOPIC DERMATITIS AND PEANUT HYPERSENSITIVITY. BURKS A W; **CONNAUGHTON C A** ; COCKRELL G E; HELM R M. LITTLE ROCK, ARKANSAS.. FORTY-EIGHTH ANNUAL MEETING OF THE AMERICAN ACADEMY OF ALLERGY AND IMMUNOLOGY, ORLANDO, FLORIDA, USA, MARCH 6-11, 1992. J ALLERGY CLIN IMMUNOL. (1992) 89 (1 PART

2), 298. CODEN: JACIBY. ISSN: 0091-6749. Language: English.

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1992:159884 Document No.: BR42:76084. THE IDENTIFICATION OF PEANUT AGGLUTININ AS A **PEANUT ALLERGEN** IN PATIENTS WITH ATOPIC DERMATITIS AND PEANUT HYPERSENSITIVITY. BURKS A W; **CONNAUGHTON C A** ; COCKRELL G E; HELM R M. UNIV. ARKANSAS MED. SCI., LITTLE ROCK, ARKANSAS, USA.. *JOINT MEETING OF THE SOUTHERN SOCIETY FOR CLINICAL INVESTIGATION, THE SOUTHERN SECTION AMERICAN FEDERATION FOR CLINICAL RESEARCH, THE SOUTHERN SOCIETY FOR PEDIATRIC RESEARCH, AND THE SOUTHERN REGION SOCIETY FOR INVESTIGATIVE DERMATOLOGY, NEW ORLEANS, LOUISIANA, USA, JANUARY 29-31, 1992.* CLIN RES. (1991) 39 (4), 852A. CODEN: CLREAS. ISSN: 0009-9279. Language: English.

L28 ANSWER 50 OF 50 MEDLINE DUPLICATE 15

91349427 Document Number: 91349427. PubMed ID: 1880317. Identification of a major **peanut allergen**, Ara h I, in patients with atopic dermatitis and positive peanut challenges. Burks A W; Williams L W; Helm R M; **Connaughton C**; Cockrell G; O'Brien T. (University of Arkansas for Medical Sciences, Little Rock.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1991 Aug) 88 (2) 172-9. Journal code: H53; 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB Peanuts are among the most common causes of immediate hypersensitivity reactions to foods. Serum from nine patients with atopic dermatitis and a positive double-blind, placebo-controlled, food challenge to peanut were used to begin the process of identification and purification of the major **peanut allergens**. Identification of a major **peanut allergen** was accomplished by use of anion-exchange column chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, ELISA, thin-layer isoelectric focusing, and IgE-specific immunoblotting. Anion-exchange chromatography revealed several fractions that bound IgE from the serum of the challenge-positive patient pool. By measuring antipeanut-specific IgE in the ELISA and in IgE-specific immunoblotting, we identified an allergenic component with two Coomassie brilliant blue staining bands by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a mean molecular weight of 63.5 kd. Examination of this fraction by the IgE antipeanut ELISA with individual serum and by the ELISA-inhibition assay with pooled serum, we identified this fraction as a major allergen. Thin-layer isoelectric focusing and immunoblotting of this 63.5 kd fraction revealed it to have an isoelectric point of 4.55. Based on allergen nomenclature of the IUIS Subcommittee for Allergen Nomenclature, this allergen is designated, Ara h I (Arachis hypogaea).

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Engineering, Characterization and in vitro Efficacy of the Major Peanut Allergens for Use in Immunotherapy

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Departments of [a]Biochemistry and Molecular Biology and [b]Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, Ark., and [c]Department of Pediatrics, Mt. Sinai School of Medicine, New York, N.Y., USA

Key Words

Hypoallergenic · Allergens · Immunotherapy · Peanut allergy

Abstract

Background: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. ***Methods:*** The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. ***Results:*** In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. ***Conclusions:*** These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.

Introduction

In North America and Europe a large portion of the population is affected by allergic reactions to common environmental proteins, making atopic disorders among

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the most prevalent diseases in the industrialized nations [1]. In the most severe cases, exposure to an allergen in sensitive individuals can produce a life-threatening anaphylactic response. For example, allergy to peanuts is the most common single cause of anaphylaxis outside of hospitals in the United States [2, 3].

Documented food hypersensitivity reactions affect approximately 6–8% of children and 1–2% of adults [4, 5]. Peanuts, milk and eggs account for the majority of reactions in children, while peanuts, tree nuts and shellfish are responsible for the majority of reactions in adults [6]. Most children will outgrow allergies to foods such as milk, eggs, wheat and soybean. Unfortunately, allergy to peanuts will typically persist into adulthood, lasting the entire lifetime of the individual. It is estimated that about 0.6% of the United States population is allergic to peanuts [7].

Despite the prevalence of peanut hypersensitivity in the population, and an increasing number of deaths each year from peanut-induced anaphylaxis, there are no therapeutic options available to prevent life-threatening allergic reactions to peanut. The only treatment available for peanut-hypersensitive patients is strict avoidance of peanut allergens [2]. Recently, the food industry has started using peanuts as a supplemental protein source in a wide variety of processed foods [8]. This inclusion of potential hidden peanut allergens makes accidental consumption almost unavoidable for peanut-sensitive individuals. In fact, in a retrospective study of peanut-induced clinical symptoms, 50% of allergic individuals suffered a peanut-induced hypersensitivity reaction in a 2-year period [9].

In this communication, we describe the characteristics of hypoallergenic variants of the major peanut allergens Ara h 1, Ara h 2 and Ara h 3 that exhibit properties favorable for allergen immunotherapy. By modifying the allergen cDNAs to express proteins with reduced IgE-binding capacity, it was possible to produce hypoallergenic variants. These variants were shown to be poor competitors for serum IgE from a pool of peanut-hypersensitive patients; however, they retained the ability to stimulate T cell proliferation. In addition, desensitization of a murine model of peanut allergy with the modified Ara h 2 protein resulted in reduced amounts of Ara h 2-specific IgE and significantly lowered anaphylaxis scores when compared to controls. The modified peanut allergen genes and proteins could provide a safe immunotherapeutic agent for the treatment of peanut allergy.

Results

The ability of the modified peanut allergens to bind IgE from individual patients was determined by immunoblot analysis. Serum IgE from peanut-sensitive patients was incubated with immunoblot strips containing equal amounts (2 μg) of the wild-type and modified proteins and the amount of IgE bound to each as determined as described above. Individual patient serum IgE binding to the modified protein was expressed as a percentage of IgE binding to the wild-type allergen. There were 57 patients tested for IgE binding to Ara h 1, 52 patients for Ara h 2, and 52 patients for Ara h 3. The range of IgE binding to modified allergens was from 5.4–125% of wild-type Ara h 1 allergen, 0–99.8% of wild-type Ara h 2 allergen and 19.3–141% of wild-type Ara h 3 allergen. The average decrease in IgE binding to the modified allergen in the current population of peanut-sensitive patients was 35% for Ara h 1, 71% for Ara h 2 and 41% for Ara h 3.

To examine the ability of the modified allergens to continue to interact with T cells from peanut-sensitive individuals, peripheral blood mononuclear cells (PBMC) were incubated in the presence of these proteins and proliferation was assessed by the incorporation of radioactive thymidine into the DNA of dividing cells. PBMCs from 12 peanut-sensitive individuals were tested for each wild-type and modified allergen and the stimulation index for the modified allergen was expressed as a percentage of that obtained for the wild-type allergen. The range of stimulation indexes for the recombinant wild-type allergens was 2–28.7 for Ara h 1, 2 –12.7 for Ara h 2 and 2–26.3 for Ara h 3. In comparison, the average stimulation index produced by the modified allergens in relation to its wild-type counterpart was 72% for Ara h 1, 104.3% for Ara h 2 and 72.2% for Ara h 3. These results indicate that the modified allergens retained the ability to interact with T cells.

Utilizing a murine model of peanut anaphylaxis [10], wild-type Ara h 2 was used to first sensitize mice, and then desensitize the mice with either wild-type Ara h 2 protein or the modified Ara h 2 protein and monitor histamine release and clinical symptoms. As expected, PBS-sham-treated mice exhibited significantly higher clinical symptom scores when compared to either of the experimental groups. While the mice treated with the wild-type Ara h 2 protein appeared to experience reduced hypersensitivity symptoms compared to controls following challenge, the mice desensitized with modified Ara h 2 had significantly lower clinical symptom scores than either of these groups. Furthermore, to determine whether the

reduced clinical symptom scores in the modified Ara h 2 protein-treated mice reflected a reduced histamine release, we measured plasma histamine levels following challenge. Histamine levels were markedly increased in sham-treated mice compared to naïve mice and in the modified Ara h 2-treated groups.

Discussion

Our results suggest that it is possible to engineer a hypoallergenic protein that fulfills important criteria for use as an immunotherapeutic agent for allergic disease. A lowered capacity to bind IgE allows higher doses of allergen to be used, a criterion for successful immunotherapy [11], with lowered probability of life-threatening side effects. Retention of the allergen's ability to interact with T cells allows for the shift from an allergen-specific Th2 response to an allergen-specific Th1-type response. By injecting larger quantities of modified peanut allergens, there is a better chance that these proteins would be processed by nonprofessional APCs. If processed peptides are presented to circulating T cells by APCs without the proper costimulatory molecules, then T cells will become desensitized to that T cell epitope [12]. Desensitization of the T cell results in a decrease in IL-4 production, resulting in a decreased capacity to activate B cells to produce peanut allergen-specific IgE. Since the modified peanut proteins displayed a decreased capacity to bind serum IgE *and* retained the ability to stimulate T cell proliferation from PBMCs donated by peanut-allergic patients, the modified proteins have the potential to be useful in a clinical setting for allergen-specific immunotherapy aimed at treating peanut-hypersensitive patients.

Acknowledgments

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PEANUT HYPERSENSITIVITY

IgE Binding Characteristics of a Recombinant *Ara h* I Protein

J. S. Stanley, R. M. Helm, G. Cockrell, A. W. Burks, and G. A. Bannon

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Peanut allergy is a significant health problem because of the potential severity of the allergic reaction, the life-long nature of the allergic hypersensitivity, and the ubiquitous use of peanut products. Milk, eggs, and peanuts are three foods which cause over 80% of food hypersensitivity reactions in children (1,2). Unlike the food hypersensitivity reactions to milk and eggs, peanut hypersensitivity reactions usually persists into adulthood and last for a lifetime (3). Despite the prevalence of peanut hypersensitivity reactions and several fatalities annually, the identification of the clinically relevant antigens is incomplete and an understanding of the immunobiology of peanut hypersensitivity is very limited (4–6).

Recombinant methodology to clone allergens provides an efficient means of producing pure polypeptides which, in their native source, form complex mixtures and are often represented in only very small amounts (7). Several inhaled allergens have been cloned, including the allergens of house dust mites (8) and pollen grains (9,10), in comparison little work has been directed toward producing recombinant food allergens. Because of the prevalence and severity of peanut hypersensitivity reactions in both children and adults, coupled with the recent identification of two peanut allergens (*Ara h* I and *Ara h* II) that are involved in this process (11,12), we set out to clone and characterize the *Ara h* I peanut allergen. Using serum IgE from peanut hypersensitive individuals, IgE reactive clones were isolated from a peanut cDNA expression library. *Ara h* I clones were then selected from this group of potential recombinant allergens by probing with a ^{32}P-labeled *Ara h I* PCR clone constructed by amplifying peanut mRNA with an *Ara h* I oligonucleotide and oligo dT (Fig. 1). After identification of a full-length *Ara h* I cDNA clone, the frequency of IgE binding by individual patients sera to the recombinant protein and purified, native *Ara h* I from whole peanut extracts was determined by immunoblot analysis. Of the 18 patients tested in this manner, 17 had IgE which recognized recombinant *Ara h* I (Table I). In general, there was good agreement between the level of IgE binding of recombinant and native *Ara h* I for each individual. For example, patients who had high levels of IgE binding to native allergen also showed high immunoreactivity with recombinant *Ara h* I

New Horizons in Allergy Immunotherapy
edited by Sehon et al. Plenum Press, New York, 1996

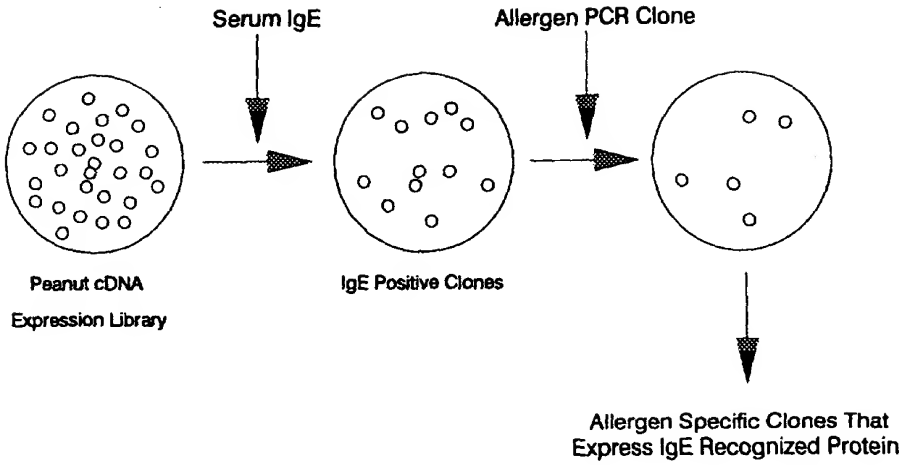

Figure 1. Strategy for isolation of *ARA h* I cDNA clones that produce IgE recognized proteins. Using Serum IgE from peanut hypersensitive individuals, IgE reactive clones were isolated from a peanut cDNA expression library as described by Burks et al. (13). *Ara h I* was then selected from this group of potential recombinant allergens by probing with a ^{32}P labeled *Ara h I* PCR clone.

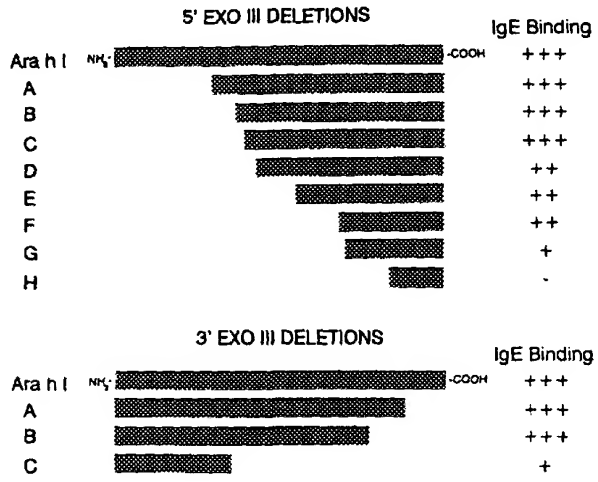

Figure 2. EXO III deletions of the intact *ARA h* I cDNA clone indicate multiple IgE binding domains. Exo III digestion from the 5' or 3' end of the full length *Ara h* I cDNA clone was used to produce shortened clones whose protein products could then be tested for IgE binding by immunoblot analysis. The pluses (+) on the right side of this figure indicate the extent of IgE binding to the protein product of each construct. All constructs bound IgE until they were reduced to the extreme carboxyl terminal (5' Exo III) or amino terminal (3' Exo III) end of the molecule. These results indicate that there are multiple IgE epitopes on the *Ara h* I allergen.

Table 1. Comparison of serum IgE binding to native and recombinant *Ara h* I protein

Patient	Native *Ara h* I	Recombinant *Ara h* I
1	+++	+++
2	+++	+++
3	+++	+++
4	+++	+++
5	+++	+++
6	++	+++
7	+++	+++
8	++	++
9	++	++
10	+	+
11	+	−
12	+	+
13	+	+
14	+	+
15	+	+
16	+	+
17	+	+
18	+	+
19	−	−

Purified *Ara h* I from whole peanut extracts or recombinant *Ara h* I protein was electrophoresed on denaturing polyacrylamide gels, blotted to nitrocellulose, and then probed with serum IgE from patients with peanut hypersensitivity (A-R) or serum IgE from an individual who is not peanut allergic (S). Patients were scored for the presence (+) or absence (−) of serum IgE to recombinant or native *Ara h* I.

protein. Patients who had low levels of IgE binding to native allergen showed low reactivity with the recombinant protein. Only one peanut sensitive individual who had serum IgE specific to native *Ara h* I had no detectable IgE which recognized the recombinant protein.

Since it appeared that the recombinant *Ara h* I protein bound IgE with the same degree/intensity as native allergen, we set out to map the major IgE binding domain(s) on the recombinant molecule. Exo III digestion from the 5' or 3' end of the full length *Ara h* I cDNA clone was used to produce shortened clones whose protein products could then be tested for IgE binding by immunoblot analysis (Fig.2). The pluses (+) on the right side of this figure indicate the extent of IgE binding to the protein product of each construct. All constructs bound IgE until they were reduced to the extreme carboxyl terminal (5' Exo III) or amino terminal (3' Exo III) end of the molecule. These results indicate that there are multiple IgE epitopes on the *Ara h* I allergen. These results are significant in that they indicate the utility of using recombinant peanut allergens for studying peanut hypersensitivity.

ACKNOWLEDGMENTS

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Structure of the Major Peanut Allergen Ara h 1 May Protect IgE-Binding Epitopes from Degradation[1]

Soheila J. Maleki,[2]* Randall A. Kopper,† David S. Shin,† Chun-Wook Park,* Cesar M. Compadre,‡ Hugh Sampson,§ A. Wesley Burks,* and Gary A. Bannon[3]*†

In the past decade, there has been an increase in allergic reactions to peanut proteins, sometimes resulting in fatal anaphylaxis. The development of improved methods for diagnosis and treatment of peanut allergies requires a better understanding of the structure of the allergens. Ara h 1, a major peanut allergen belonging to the vicilin family of seed storage proteins, is recognized by serum IgE from >90% of peanut-allergic patients. In this communication, Ara h 1 was shown to form a highly stable homotrimer. Hydrophobic interactions were determined to be the main molecular force holding monomers together. A molecular model of the Ara h 1 trimer was constructed to view the stabilizing hydrophobic residues in the three dimensional structure. Hydrophobic amino acids that contribute to trimer formation are at the distal ends of the three dimensional structure where monomer-monomer contacts occur. Coincidentally, the majority of the IgE-binding epitopes are also located in this region, suggesting that they may be protected from digestion by the monomer-monomer contacts. On incubation of Ara h 1 with digestive enzymes, various protease-resistant fragments containing IgE-binding sites were identified. The highly stable nature of the Ara h 1 trimer, the presence of digestion resistant fragments, and the strategic location of the IgE-binding epitopes indicate that the quaternary structure of a protein may play a significant role in overall allergenicity. *The Journal of Immunology,* **2000, 164: 5844–5849.**

Legume seed storage proteins constitute the third largest source of dietary protein on Earth. They are of particular importance as a nutritional source in developing countries that lack ample supplies of animal protein (1). Peanuts are widely used for the preparation of a variety of foods in the U.S. and are also relied on as a protein extender in developing countries. There has been an increase in the observed incidence of peanut allergies in children over the last 10 years. This is thought to be due to the increased popularity and use of peanut products by the population in the last decade and the introduction of peanut products to children's diets at an early age (2–5). Thus, it is increasingly common for the public to be exposed to an abundantly utilized and often disguised food such as peanuts. This has led to increasing rates of sensitization, accidental ingestion, anaphylaxis, and even death in peanut-allergic patients.

There are a number of characteristics that increases the capacity of a food allergen to provoke a dangerous systemic allergic reaction. These include its ability to stimulate high titers of IgE and to resist gastrointestinal degradation sufficiently to produce fragments containing multiple IgE binding epitopes. The more degraded an allergen becomes, the more fragments are produced that contain single IgE-binding epitopes. Protein fragments containing single IgE-binding sites are incapable of cross-linking IgE-bound FcεR1 receptors and therefore of causing mast cell degranulation. Thus, the biochemical and structural aspects of allergens play a critical role in the disease process.

The general biochemical characteristics of most food allergens indicate that they are low m.w. glycoproteins (<70 kDa) with acidic isoelectric points that are highly abundant in food. These proteins are usually resistant to proteases, heat, and denaturants, allowing them to resist degradation during food preparation and digestion (6, 7). Several studies have shown that the most allergenic portion of the peanut is the protein fraction of the cotyledon (8-10). The peanut allergen Ara h 1 is a vicilin-like seed storage protein found in the cotyledon. This protein is one of the main storage proteins of the seed and is utilized as a nitrogen and amino acid source during development of a new peanut plant. In addition to its importance to the developing plant, Ara h 1 is recognized by serum IgE from >90% of peanut-sensitive patients, thus establishing it as an important allergen in the etiology of this disease (11, 12). The linear IgE-binding epitopes of this allergen have been mapped and shown to consist of 23 independent binding sites (13). Individual patients with IgE Abs to Ara h 1 have been shown to have IgE that recognizes multiple epitopes on the Ara h 1 protein (13). These sites are evenly distributed along the linear sequence of the molecule. However, a molecular model of the tertiary structure of the Ara h 1 protein shows that the IgE-binding sites were clustered into two main regions. In addition, Ara h 1 forms homotrimers, a physical characteristic that may be important in establishing it as an allergen (14).

Using fluorescence anisotropy, we have shown that the formation of a highly stable Ara h 1 homotrimer is mediated primarily through hydrophobic interactions. A molecular model of the Ara h 1 trimer indicates that hydrophobic residues on α-helical bundles

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located on the ends of each monomer contribute to the stability of the structure. The IgE-binding epitopes are clustered in the regions of monomer-monomer contact. The importance of the trimer structure to the overall allergenicity of the molecule was investigated using assays designed to determine the stability of Ara h 1 to digestion with proteases. In this report, when Ara h 1 was incubated with digestive enzymes, various protease-resistant fragments containing IgE-binding sites were obtained, indicating that the quaternary structure of Ara h 1 may play an important role in protecting these epitopes from digestive enzymes.

Materials and Methods

Purification of peanut allergen Ara h 1

Twenty grams of crude peanut extract were added to 500 ml extraction buffer (50 mM Tris-Cl (pH 8.3), 5 mM DTT, 1 mM EDTA, 1 mM PMSF) containing 200 mM NaCl. In this report, the extraction buffer contains the chemicals indicated within the parentheses, but the salt concentration is varied as indicated for different experiments. The solution was stirred gently at room temperature, cleared by centrifugation at 13,000 × *g* for 30 min at 4°C, and subjected to ammonium sulfate precipitation (15). Ammonium sulfate was added to 70% saturation. The remaining supernatant was then taken to 100% ammonium sulfate saturation and the Ara h 1 protein collected by centrifugation. The pellet was resolubilized in extraction buffer (pH 8.3) by sonication on ice at 40% power using a Heat Systems Disruptor (Fischer Scientific, Atlanta, GA). After sonication, the solubilized proteins were desalted on disposable PD-10 gel filtration columns (Pharmacia Biotech, Piscataway, NJ) and loaded onto a High Prep S, cation exchange resin column (2.5 × 12 cm, Bio-Rad Laboratories, Hercules, CA). A linear salt gradient (200–800 mM NaCl) was used to elute Ara h 1 from the column and 2.5-ml fractions were assayed for Ara h 1 content by a 12% SDS-polyacrylamide gel (Novex, San Diego, CA) and Coomassie Brilliant Blue staining. Fractions containing Ara h 1 were pooled and desalted into desired buffers on PD-10 columns just before use in all experiments. The desired buffers used were identical with extraction buffer containing 100 mM NaCl with varying pH values (pH 8.3 for tryptic and chymotryptic digestion reactions and pH 2 for pepsin digestion for the digestion reactions). Protein concentrations were monitored using the Bio-Rad protein assay reagent kit (Bio-Rad Laboratories). At each stage, samples were subject to electrophoresis on 12% SDS-polyacrylamide gels (Novex), and the purity of the Ara h 1 fractions was assessed by Coomassie staining and densitometry of the resulting protein bands. Purified Ara h 1 was stored in aliquots at −80°C.

Serum IgE

Serum from 15 patients with documented peanut hypersensitivity reactions (mean age, 25 yr) was used to identify Ara h 1 during purification. The patients had either a positive double-blind, placebo-controlled food challenge or a convincing history of peanut anaphylaxis (laryngeal edema, severe wheezing, and/or hypotension (16). Equal aliquots of IgE-containing serum from 12 to 15 patients was pooled and used for our experiments. Each patient's serum contained IgE that recognized Ara h 1. All studies were approved by the Human Use Advisory Committee at the University of Arkansas for Medical Sciences.

Cross-linking reactions

Purified Ara h 1 was desalted into PBS, and two identical samples were diluted to a final concentration of 3 μM. The pH was adjusted with HCl to either pH 2 or pH 7.6, and the solution was allowed to incubate at room temperature for 1 h. After incubation, the Ara h 1 sample at pH 2 was adjusted to pH 7.6, and both samples were then subjected to cross-linking. The process of pH adjustment and cross-linking was minimized (<2 min) to prevent reequilibration of Ara h 1 monomers and trimers before cross-linking. The protein cross-linking reagent, dithiobis(succinimidylpropionate) (Pierce, Rockford, IL) was used. Dithiobis(succinimidylpropionate) was dissolved in *N,N*-dimethylformamide as a 74 mM stock solution. A 1-μl sample of cross-linker stock solution was added to 50 μl of the Ara h 1 samples. The reactions were quenched after 80 s by addition of SDS sample buffer (lacking DTT), heated to 100°C for 5 min, and subjected to SDS-PAGE. Limited cross-linking was performed to minimize the formation of nonspecific complexes.

Protease digestions

Purified Ara h 1 was desalted and diluted to a final concentration of 8 μM in extraction buffer containing 100 mM NaCl adjusted with HCl to pH 8.3 for tryptic and chymotryptic digestions and pH 2.0 for pepsin digestion (all proteases were purchased from Sigma (St. Louis, MO)). The diluted Ara h 1 was incubated in the presence of 0.5 μg/ml of trypsin, chymotrypsin, or pepsin at 37°C, and aliquots were taken at 0 min, 10 s, and 1, 2, 4, 8, 15, 30, 60, 120, and 180 min. The digestion reaction in each aliquot was quenched by the addition of SDS-sample buffer. Samples were then subjected to SDS-PAGE and either stained or transferred to nitrocellulose for immunoblot analysis using pooled serum from peanut allergic individuals.

Immunoblot analysis

For the detection of Ara h 1 or IgE-binding fragments of Ara h 1, immunoblot analysis was performed using serum IgE from a 15-person pool of peanut-allergic individuals. SDS-PAGE (12%)-resolved proteins were transferred to nitrocellulose membrane (0.45 μm, Schleicher and Schuell, Keene, NH) electrophoretically. The membranes were blocked in Tris-buffered saline with Tween 20 (TBST) plus 1% BSA for 2 h at room temperature. The membrane was then washed in TBST and incubated with a 1:10 dilution of pooled human sera for 1 h. Detection of the bound IgE was accomplished using ^{125}I-labeled anti-human IgE secondary Ab (Sanofi, Chaska, MN) and subsequent exposure to x-ray film.

Fluorescein labeling

Ara h 1 was desalted into $NaHPO_4$ buffer, pH 8, and labeled with FITC according to the methods described by Fernando and Royer (17) for dansyl labeling. Briefly, the FITC was dissolved in *N,N*-dimethylformamide at 250 mg/ml to make the stock solution, and 10 μl of the solution were added to 1 ml of Ara h 1 solution. This mixture was then incubated at room temperature for 10 min. The free FITC was separated from the labeled protein using a desalting column (described above) that was preequilibrated in binding buffer (10 mM HEPES/KOH (pH 7.9), 1 mM EDTA, 1 mM DTT, 5% glycerol).

Fluorescence anisotropy measurements of Ara h 1

All fluorescence measurements were made using a Beacon fluorescence polarization spectrometer (Pan Vera, Madison, WI) with fixed excitation (490 nm) and emission (530 nm) wavelengths. Fluorescence measurements were done at room temperature (24°C) in previously described binding buffer containing different salt concentrations from 0 to 1.8 M NaCl, in a final volume of 1.1 ml. Fluorescence anisotropy is described in detail by Fernando and Royer (17). A constant amount of fluorescein-labeled Ara h 1 protein (10 nM) in binding buffer was mixed with serial dilutions (by 0.5 or 0.8 increments) of unlabeled Ara h 1 to analyze oligomer formation at room temperature. Each data point is an average of three independent measurements, and each curve is fitted to a sigmoidal function using Origin (Microcal Software, Northampton, MA). Analysis of the slope of the binding curves and midway point between top and bottom plateaus allowed the calculation of the cooperativity values (ρ) and apparent dissociation constants (K_{app}) for Ara h 1 oligomer formation (18). In the equation used by Origin program for fitting a sigmoidal curve, the K_{app} is defined as the midway point between the lower and upper plateaus and the slope of the curve (ρ value) defines the cooperativity of the interaction. This equation is described in detail by Czernik et al. (18). The intensity of fluorescence remained constant throughout the anisotropy measurements.

Homology-based model of Ara h 1 trimer

A homology-based model of an Ara h 1 monomer (14) was used to construct the trimeric model. Several small areas representing Ara h 1 amino acid residues Leu^{166}-Val^{193}, Pro^{226}-Phe^{240}, Asp^{249}-Asp^{259}, and Arg^{300}-Arg^{327} were omitted during trimer construction because of structural uncertainty. The coordinates of the Cα atoms, of Asn^{48}, Ile^{117}, Phe^{174}, Glu^{202}, Ala^{312}, and Gly^{362} from the x-ray crystal structure of phaseolin[4] (Protein Data Bank code 2PHL) were used as reference points to fit the Cα atoms of Asn^{37}, Ile^{100}, Phe^{158}, Glu^{204}, Ala^{35}, and Gly^{396} from Ara h 1 to form a trimer. The Fit Monomers program from SYBYL (version 6.3, Tripos, St. Louis MO) was used to create the initial framework. The energy of the Ara h 1 trimer was minimized with a harmonic force constraint of 100 using the Charmm force field and the adopted basis Newton-Raphson method for 500 iterations using the Charmm Minimization program resident in QUANTA (version 96, Molecular Simulations, Burlington, MA). The stereochemical quality of the model was assessed by using PROCHECK (version 2.1.4, Oxford Molecular, Palo Alto, CA). The computations were performed on a Silicon Graphics workstation running IRIX 6.4.

[4] The atomic coordinates for the crystal structure of phaseolin can be accessed through the Brookhaven Protein Data Bank under PDB 2PHL (19).

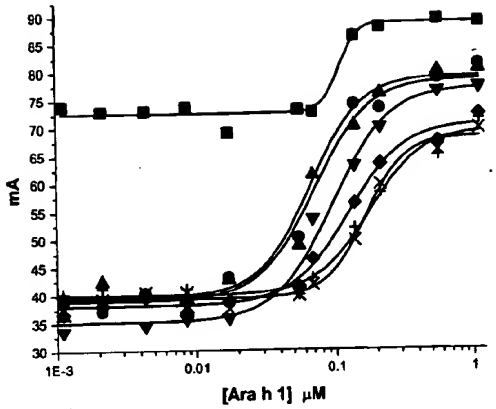

FIGURE 1. The Ara h 1 trimer is a highly stable structure due to the interaction of monomers through hydrophobic bonds. Fluorescence anisotropy was used to follow the formation of Ara h 1 higher order structure. A constant amount of fluoroscein-labeled Ara h 1 (10 nM) were mixed with serial dilutions of unlabeled Ara h 1 (x-axis) and different concentrations of NaCl. Fluorescence measurements are expressed as arbitrary millianisotropy units (mA) on the *y*-axis. Each data point represents the average of three independent measurements. ■, 0 mM NaCl; ●, 100 mM NaCl; ▲, 300 mM NaCl; ▼, 500 mM NaCl; ♦, 900 mM NaCl; +, 1.4 M NaCl; ×, 1.8 M NaCl.

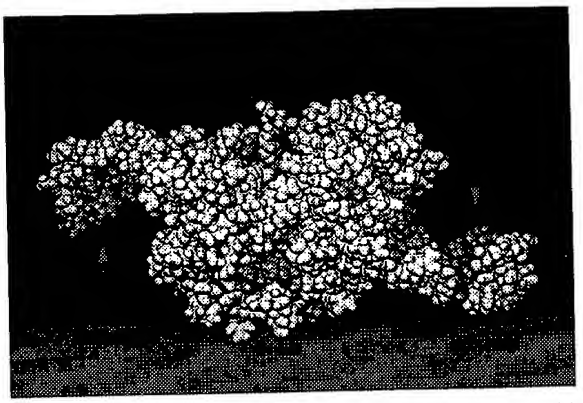

FIGURE 2. The ends of the Ara h 1 molecule contain the majority of the surface accessible hydrophobic amino acid residues. This figure shows a space filled view of the outer surface of an Ara h 1 monomer. The atoms of the hydrophobic amino acids (alanine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, valine) are shown in orange, and the remaining atoms are shown in white. Arrows, areas on the Ara h 1 monomer with the highest concentrations of surface-accessible hydrophobic amino acids.

Results

Ara h 1 trimers are stable at high concentrations of NaCl

Fluorescence anisotropy and molecular modeling methods were used to gain a better understanding of the structural properties of Ara h 1 that may contribute to its stability and allergenicity. The Ara h 1 protein is known to form a homotrimer at relatively low concentrations, and this structure has been suggested to be important to the overall allergenicity of the molecule (14). To determine the stability and types of monomer interactions that mediate the formation of a homotrimer, fluorescence anisotropy measurements were performed in the presence of increasing salt concentrations (0–1.8 M NaCl). Purified, fluorescein-labeled Ara h 1 (10 nM) was mixed with various concentrations of unlabeled Ara h 1. The anisotropy of fluorescence observed at each concentration was determined and plotted as milli-anisotropy U vs the concentration of Ara h 1 (Fig. 1). The ability of the trimer to form at NaCl concentrations as high as 1.8 M is indicated by the presence of the plateau observed at Ara h 1 concentrations of 0.1–0.3 μM. There is an increase in the dissociation constant as indicated by the increase in K_{app} from 0.065 to 0.170 μM as the NaCl concentration is increased from 0.1 M to 1.8 M. The cooperativity (ρ) of trimer formation also decreased from 2.4 to 2.1 over the same salt concentrations (Table I). Minimal transition is seen in the anisotropy curve on dilution of Ara h 1 in the absence of NaCl. This result is most likely due to accentuated oligomerization of the protein in the absence of counterions in the solution; therefore, only minimal dissociation is seen on dilution of the protein. Thus, due to the minute change in anisotropy the K_{app} and ρ values cannot be obtained with the fitting program used (see *Materials and Methods*). Collectively, these results indicate that although some ionic interactions are involved in the cooperative interaction of monomers, the formation and stability of the Ara h 1 trimer are primarily due to hydrophobic interactions.

Table 1. *Summary of association* (K_{app}) *and cooperativity* (ρ) *values for Ara h 1 trimer formation*

NaCl Concentration (mM)	K_{app}[a] (μM)	p[a]
0		
100	0.065	2.40
300	0.070	2.30
500	0.095	2.10
900	0.120	2.10
1400	0.170	2.20
1800	0.170	2.10

[a] Values were determined as described in *Materials and Methods*.

Molecular model of the Ara h 1 trimer and location of the IgE-binding epitopes

Because we had demonstrated that hydrophobic interactions were primarily responsible for Ara h 1 trimer formation, we examined the location of any surface-accessible hydrophobic amino acid residues present on the Ara h 1 monomer that may contribute to trimer formation. To accomplish this, a homology-based model of Ara h 1 tertiary structure, representing aa 172–586 (14), was utilized. The tertiary structure of the molecule consists of four domains: an α helical bundle on one end, two sets of opposing antiparallel β sheets and a α helical bundle on the opposite end. The space-filled model of the Ara h 1 molecule and the position of the hydrophobic amino acid residues (alanine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, valine) are shown in Fig. 2. The distal regions of the molecule contain the majority of the surface-accessible hydrophobic amino acids.

To construct the model, three Ara h 1 monomers were aligned to the corresponding monomers that form the phaseolin trimer,[4] a vicilin protein the x-ray crystal structure of which revealed a trimer (19). The quality of the model was assessed using the protein health module of QUANTA and PROCHECK version 2.1.4 and compared with the quality of the phaseolin trimer (19). Most of the backbone torsion angles for nonglycine residues lie within the allowed regions of the Ramanchandran plot (data not shown). Only 1.0% of the amino acids in the Ara h 1 trimer have torsion angles that are disallowed as compared with 0.3% of amino acids in phaseolin. Side chain parameters, χ-1 and χ-2 angles, were also tested in the Ara h 1 trimer. The majority of the side chains are within the ideal 2.5 SD range where the percentage of residues

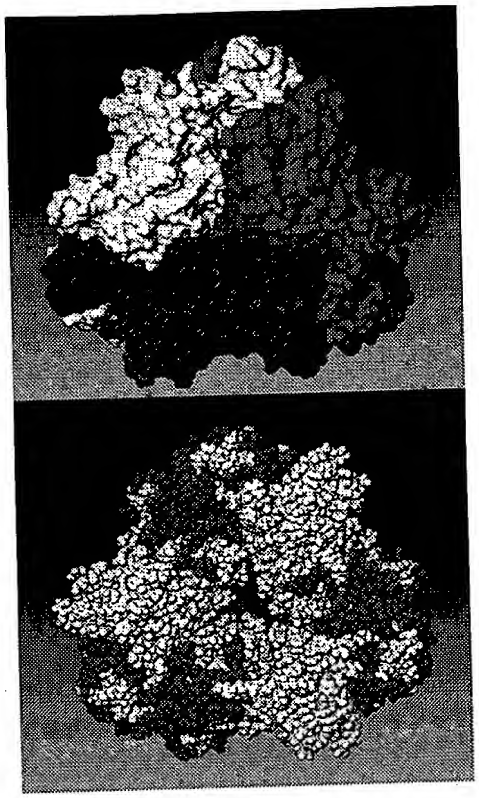

FIGURE 3. Molecular model of the Ara h 1 trimer. *Top*, Solid Connolly surface depiction of the Ara h 1 trimer. The α helical bundles located on the ends of each monomer overlap to form the trimer. *Bottom*, space-filled model of the Ara h 1 trimer with IgE-binding epitopes 10–22 colored in red, with the rest of the atoms in white. The majority of the epitopes lie near the areas of monomer-monomer contact.

outside the criteria is 19.3% for the Ara h 1 trimer and 16.9% for the phaseolin trimer. In addition to these criteria, a variety of main chain parameters (Omega angles, Van der Waals contacts, ζ angles, and H-bond energy) were tested for stereochemical quality and were comparable with that of the phaseolin x-ray crystal structure. Taken together, these data indicate that the homology-based model of the Ara h 1 trimer is reasonable and similar to the phaseolin trimer. The global fold of the Ara h 1 trimer indicates that formation of this structure is due to the α helical bundles on the ends of one Ara h 1 monomer overlapping with those of the adjacent monomer (Fig. 3, *top*). The hydrophobic residues depicted in Fig. 2 are those that form the interface between the monomers, similar to the structure of the phaseolin trimer (19).

The majority of the IgE-binding epitopes (13) are clustered near the regions of Ara h 1 monomer-monomer contact (Fig. 3, bottom). Epitopes 11 and 12 on the α helical bundle of one monomer and epitopes 20 and 21 on the α helical bundle of another monomer contact one another when the trimer is formed. Whereas most of the amino acid residues contained within these epitopes are surface accessible in the Ara h 1 monomer, ~40% of the residues within these epitopes lose surface accessibility when the trimer is formed as calculated by the QUANTA molecular simulation program.

Digestion-resistant fragments containing intact IgE epitopes

To determine whether quaternary structure played any role in protecting the Ara h 1 molecule from proteolytic digestion it was essential to determine whether the Ara h 1 trimer would remain

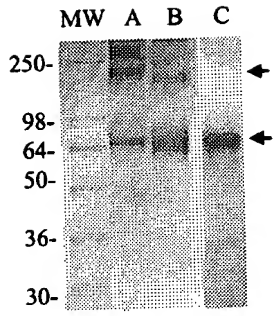

FIGURE 4. The Ara h 1 trimer is stable at pH 2 for 1 h. Ara h 1 trimers were incubated at pH 2 for 1 h and then subjected to a cross-linking reaction. The amount of covalently stabilized Ara h 1 trimer produced under these conditions was assessed by SDS-PAGE analysis. *Lane MW*, MW standards; *Lane A*, Ara h 1 trimers cross-linked after 1 h of incubation at pH 7.6; *Lane B*, Ara h 1 trimers cross-linked after 1 h of incubation at pH 2.0; *C*, Ara h 1 protein without any cross-linking. Arrows, Ara h 1 monomers and trimers.

intact when exposed to the environment of the stomach. Therefore, purified Ara h 1 was exposed to acidic pH. At the end of this incubation period, a cross-linking reaction was performed, and the amount of Ara h 1 trimer formed under these conditions was visualized by SDS-PAGE analysis (Fig. 4). Even though effects of acid hydrolysis can be seen on the integrity of the protein, it is clear that the Ara h 1 oligomer was found to be stable even after incubation at pH 2 and could still bind IgE (data not shown).

According to anisotropy experiments, at concentrations above 300 μM, Ara h 1 is in the form of a highly stable trimer. To determine whether some of the IgE binding epitopes were protected from digestion as predicted by the quaternary structure determinations, Ara h 1 was exposed to trypsin, chymotrypsin, or pepsin; proteases encountered in the gastrointestinal tract. Protease concentrations were such that nonallergenic proteins tested were digested to small (<10 kDa) peptides in a short period of time (<1 h, data not shown). The Ara h 1 peptides produced by these digestions were subject to SDS-PAGE and visualized by Coomassie staining. Peptides ranging in size from 16 kDa to 29 kDa were observed on Coomassie-stained gels up to 3 h after the start of digestion (Table II). A representative digestion of Ara h 1 with chymotrypsin has been shown in Fig. 5 as an example. The data from digestions with pepsin, trypsin, and chymotrypsin have been summarized in Table II. These peptide fragments ranged in size

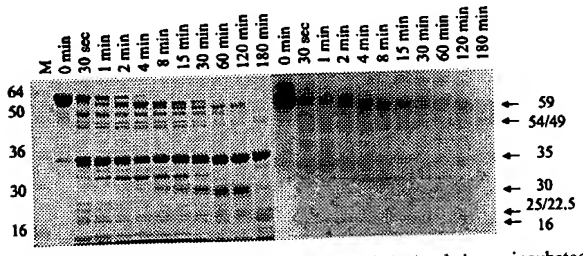

FIGURE 5. Chymotrypsin digestion of Ara h 1. Ara h 1 was incubated with chymotrypsin for the various time points indicated. Samples were run on SDS-PAGE and stained with Coomassie (*left*). The same digestion samples were transferred to nitrocellulose and analyzed by Western blot analysis using serum from allergic individuals. The bound serum IgE was recognized by an ^{125}I-anti-human secondary Ab and subject to autoradiography (*right*). Arrows, location and size of the chymotrypsin-resistant fragments that are recognized by serum IgE.

Table II. *Protease-resistant Ara h 1 peptides*

Pepsin			Trypsin			Chymotrypsin		
Digestion time (min)	MW[a] (kDa)	IgE binding	Digestion time (min)	MW[a] (kDa)	IgE binding	Digestion time (min)	MW[a] (kDa)	IgE binding
0.5–15	62		1–15	61		0.5–30	62	
0.5–15	60	***	8–15	58		2–180	59	***
0.5–30	50	***	0.5–120	50	***	0.5–180	54	***
0.5–30	47.5		0–60	36	***	0.5–180	49	***
0.5–30	45		1–60	35	***	0.5–180	48	
0.5–30	43		2–120	30		0.5–180	35.5	***
2–30	35.5	***	4–180	28	***	0.5–180	34	
2–30	33	***	8–180	26	***	0.5–120	32.5	
0.5–15	31		4–180	23		4–180	30	***
0.5–30	29		4–180	18.5		8–180	29	
0.5–180	27	***	30–180	17		0.5–180	26	
2–180	26.5	***	0.5–8	16		0.5–180	25	***
4–180	23.5	***	4–15	15		1–30	22.5	***
8–180	22.5	***				1–180	16	***

[a] MWs of peptides were established from MW standards on SDS-PAGE.
***, IgE binding fragments.

from 16 to 61 kDa during 3 h of digestion. To determine whether the long lived peptides contained IgE binding epitopes, the Ara h 1 digestion products were subjected to immunoblot analysis using serum IgE from a pool of peanut-sensitive patients. In each case, many peptides contained intact binding sites that could be recognized by serum IgE (Table II). Knowing that Ara h 1 contains 23 IgE binding sites that are evenly distributed along the linear sequence of the molecule (13), these results suggest that large proteolytic fragments of Ara h1 contain multiple IgE-binding epitopes and survive digestion by the gastrointestinal enzymes tested.

Discussion

In the past, the study of allergens at a molecular level has been largely limited to biochemical measurements such as size, isoelectric points, glycosylation, and resistance to denaturation and digestion (6, 20–23). Ara h 1 has all of the classic characteristics of a food allergen. It is a 65-kDa glycoprotein with an acidic isoelectric point. It is an abundant protein in the peanut (11) that survives food-processing methods intact (6) and is stable up to 1 h within the in vitro digestion systems designed to mimic the gastrointestinal tract (6). Resistance of allergens to digestive enzymes have been attributed to various factors including protease inhibitors or nonprotein components present in the extracts analyzed (7, 24), direct effects on the secretion of endogenous proteins and/or the structure of the allergen itself (25). To date, there has been limited information regarding the structural basis for the stability and resistance of an allergen to digestion. In addition, the nature of the digestion-resistant fragments, especially the IgE-binding characteristics of these fragments have not been determined for any allergen. The combination of molecular biology, fluorescence anisotropy, and protein computer modeling has allowed us to examine a new set of important allergen characteristics. We have been able to identify IgE-binding sites, the amino acid residues critical for IgE binding (14), monomer tertiary structure, and oligomer formation. In this communication, we have examined the biochemical forces involved in oligomerization, identified the hydrophobic amino acids critical for this interaction, and shown that the locations of these residues coincide with the IgE recognition sites on the tertiary structure. Digestion-resistant fragments containing multiple IgE-binding sites were identified. Together these results show that quaternary structure may play an important role in the allergenic properties of a protein.

The only therapeutic option presently available for the prevention of a food hypersensitivity reaction is food avoidance. Unfortunately, for a ubiquitous food such as peanut, the possibility of inadvertent ingestion is great (26–28). Because of the potential severity of the allergic reaction, it has been suggested that a hypoallergenic Ara h 1 gene could be developed to replace its allergenic homologue in the peanut genome, thus blunting allergic reactions in sensitive individuals who inadvertently ingest this food (14). Because the Ara h 1 gene product is such an abundant and integral seed storage protein, it would be necessary for the altered vicilin to retain as much of its native function, properties, and three-dimensional structure as possible. The data presented here indicate that most of the IgE-binding epitopes are located at the contact points between the monomers in the Ara h 1 homotrimer. Amino acid substitutions designed to reduce the allergenicity of this protein may have deleterious effects on trimer formation and protein function. On the other hand, weakly destabilizing mutations may be desirable in making IgE-binding sites available to digestive enzymes.

It has been shown that Ara h 1 is capable of forming trimeric complexes in vitro similar to that of other vicilins (14). This multimeric form is also observed in the phaseolin x-ray crystal structure (19) and within the initial multiple isomorphous replacement electron density maps of canavalin[5] (29). For phaseolin, the primary site of monomer contact was overlap of the terminal α helical bundles. A trimeric model of Ara h 1 based on the phaseolin structure revealed that the α helical bundles of the Ara h 1 monomers also appear to be the primary site for monomer contact. In addition, fluorescence anisotropy experiments clearly indicate that trimer formation is primarily mediated through hydrophobic interactions; this is confirmed by the location of hydrophobic residues in the areas of monomer-monomer contact. As previously mentioned, the location of the intramolecular hydrophobic contacts coincides with the location of preponderance of the IgE-binding epitopes. Also, when examining all of the available protease recognition sites located on the Ara h 1 primary sequence, it is clear that several of these sites are protected from digestion. Considering that Ara h 1 contains 23 IgE epitopes that, when the protein is denatured, are somewhat evenly distributed along the primary amino

[5] The atomic coordinates for the crystal structure of canavalin can be accessed through the Brookhaven Protein Data Bank under PDB 1CAU (29).

acid sequence of the molecule, and the majority of the peanut-allergic individuals are known to recognize a minimum of five epitopes, it becomes obvious that any fragment larger than 10 kDa must contain more than one IgE-binding site. Collectively, these results suggest that the formation of a trimeric complex may afford the molecule some protection from protease digestion and denaturation, allowing passage of large fragments of Ara h 1 containing several intact IgE-binding epitopes across the small intestine, therefore contributing to its allergenicity.

Studies designed to develop hypoallergenic alternatives to replace native allergens in plants must take into account not only those amino acid substitutions that result in IgE binding but also those that will not disrupt the native structure of larger protein complexes. Thus, the development of an assay system that allows us to measure trimer formation and stability will permit us to test the integrity of recombinant proteins before plant transformation. Also, mutations in the recombinant protein that may allow trimer formation while having a destabilizing effect may increase the susceptibility of Ara h 1 to acid hydrolysis and digestion, therefore rendering it less likely to cause sensitization. Given the widespread use of peanuts in consumer foods and the potential risk this poses to individuals genetically predisposed to developing peanut allergy and to the health of individuals already peanut sensitive, these approaches are currently being explored in our laboratories.

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